Biochemical and Biophysical Research Communications 422 (2012) 200–205

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Clusterin induces the secretion of TNF-α and the chemotactic migration of macrophages

Young-Jun Shim [a], Byeong-Ho Kang [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 27 April 2012
Available online 7 May 2012

Keywords:
Clusterin
TNF-α
Chemotaxis
Metastasis
Macrophages

ABSTRACT

Tumor associated macrophages are known to be closely linked with tumor progression and metastasis. On the other hand, clusterin is overexpressed in several tumor types and regarded as a putative tumor-promoting factor due to this overexpression and the subsequent induction of chemoresistance. In our previous study, clusterin was found to induce the expression of matrix metalloproteinase-9 (MMP-9) in macrophages, and MMP-9 is known to be essential for tumor cell migration and invasion via basement membrane breakdown. Because paracrine interactions between tumor cells and surrounding macrophages regulate metastasis, these findings raise the possibility that clusterin promotes the secretion of cytokines in macrophages in addition to MMP-9. Here, we demonstrate that clusterin upregulates the expressions of chemotactic cytokines, that is, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1β (MIP-1β), regulated upon activation, normal T cell expressed and secreted (RANTES), and tumor necrosis factor-α (TNF-α) in Raw264.7 macrophages. In particular, clusterin stimulated TNF-α secretion via the activations of ERK, JNK, and PI3K/Akt pathways in a time and dose-dependent manner. Furthermore, clusterin-induced TNF-α secretion was found to play a critical role in the chemotactic migration of Raw264.7 macrophages. It was also found that clusterin acts directly as a chemoattractant for macrophages. Together, these results suggest that clusterin stimulates the expression and secretion of TNF-α, which plays a critical role in promoting macrophage chemotaxis, via ERK, JNK, and PI3K/Akt pathways. Collectively, these findings describe a novel function for clusterin as an inducer of TNF-α in macrophages and their chemotactic migration, and suggest that clusterin has a tumor-promoting effect.

1. Introduction

Metastasis is the most lethal aspect of malignant tumors, and a highly complex process. The propensity to progress and metastasize is a characteristic of tumor cells [1], and solid tumors consist of malignant cells in the presence of stromal cells, such as, fibroblasts, endothelial cells and immune cells and extracellular matrix (ECM) [2]. Over previous decades, it was believed that intrinsic factors in tumor cells, such as, mutations of tumor suppressor genes render tumor cells aggressive and underlie cancer progression. However, the current major focus in cancer research is on cytokine-mediated communications between tumor and stromal cells.

Macrophages are the predominant infiltrating immune cells in the tumor stromal compartment, and it is generally believed that tumor-associated macrophages (TAMs) play important roles during tumor growth, progression, and metastasis by secreting various growth factors, proteases, and cytokines, which accelerate angiogenesis, connective tissue breakdown, and tumor-cell proliferation [3]. TAMs are mainly derived from circulating blood monocytes [4] and are typically restricted to stromal areas, and their migration is known to be mediated by a number of chemotactic cytokines. TNF-α is one of these chemotactic factors, and is capable of recruiting macrophage and monocyte [5]. In addition, it has been shown that TNF-α is expressed in the microenvironments of many different types of cancer, and has been proposed to be a critical mediator that bridges inflammation and tumor progression.

On the other hand, clusterin (CLU) is a multifunctional glycoprotein that is secreted and expressed ubiquitously in all human body fluids [6]. This disulfide-linked glycoprotein has been implicated in diverse pathophysiological processes required for tumor formation and progression, including; apoptotic inhibition, chemoresistance induction, tissue remodeling, and immune system regulation [7]. Furthermore, the up-regulation of CLU protein has been

* Corresponding author. Address: Department of Pharmacology, College of Medicine, Korea University, 126-1 Anam-Dong 5 Ga, Seongbuk-Gu, Seoul 136-705, Republic of Korea. Fax: +82 2 927 9289.
E-mail address: bhmin@korea.ac.kr (B.-H. Min).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.162

reported in a wide range of advanced cancers such as breast and lung carcinoma [8,9], and the engineered overexpression of CLU has been found to render cancer cells resistant to cytokine-induced apoptosis [10] and to enhance tumor progression and metastasis [11].

In order for tumor cells to become metastatic, the rearrangement of tumor stroma is required, and MMP-9 can cleave ECM components and basement membranes, and thereby, create essential routes for invading tumor cells. In a previous study, we demonstrated that clusterin activates and induces MMP-9 expression in macrophages [12]. This finding led us to investigate whether clusterin stimulates macrophages to secret chemotactic cytokines associated with cancer metastasis, and, if so, to determine which signaling pathways are involved.

The present study shows clusterin enhances the secretions of TNF-α and a variety of chemokines, such as, MCP-1, MIP-1β, and RANTES in macrophages and that it promotes their chemotactic migration. This finding probably explains the clusterin overexpression observed in cancers and its putative tumor-promoting role. Our findings suggest that clusterin secreted by cancer cells is an important mediator (or inducer) of tumor metastasis as the clusterin-induced release of chemokines may facilitate the recruitment of macrophages or monocytes to tumor sites and contribute to the creation of a microenvironment favoring metastasis.

2. Materials and methods

2.1. Reagents and antibodies

LPS (0111:B4) and polymyxin B were from Sigma–Aldrich (St. Louis, MO). PD98059, SB203580, SP600125, and LY294002 were from Calbiochem (San Diego, CA). Phospho-specific and total form antibodies against ERK, p38, JNK, and Akt (ser473) were purchased from Cell Signaling Technology (Beverly, MA). Monoclonal anti-clusterin antibody (1G8) was generated in our laboratory.

2.2. Cell culture

Raw264.7 and peritoneal macrophages [12] were grown in DMEM and RPMI1640, respectively. Both media were supplemented with 10% FBS and 1% antibiotics-antimycotics (Gibco-BRL, Rockville, MD) in a 5% CO_2 humidified incubator at 37 °C.

2.3. Western blot analysis

Western blot assays on samples from Raw264.7 cells and peritoneal macrophages were performed under standard conditions as described previously [12], using appropriate antibodies (see Supplementary Materials and Methods).

2.4. Cytokine array

Cytokine release into conditioned media after clusterin treatment was monitored utilizing a Mouse Cytokine Array kit (ARY006) (R&D systems) according to the manufacturer's protocols. Briefly, conditioned media (8 ml) from Raw264.7 cells treated with clusterin (1 μg/ml) for 24 h were concentrated 5-fold using an Amicon Ultra centrifugal filter device. After blocking, array panels were incubated in a mixture of the conditioned media (1.6 ml) and a cocktail of biotinylated detection antibody (15 μl) overnight at 4 °C on a rocking platform. Any cytokine/detection antibody complex present is bound by its cognate immobilized capture antibody on the membrane. After a wash to remove unbound material, streptavidin-HRP and chemiluminescent detection reagents were added sequentially.

2.5. RNA isolation and RT-PCR

RAN isolation and RT-PCR was performed as described previously [12], using the specific primers (see Supplementary Materials and Methods).

2.6. Transwell filter assay

Raw264.7 (2×10^5) cells suspended in 200 μl of serum-free DMEM were overlaid onto transwell 8-μm pore filters in a 24-well plate format (Becton Dickinson, CA). The lower chambers contained the concentrated clusterin-treated Raw264.7 media (500 μl) containing pre-incubated TNF-α neutralizing antibody (10 μg/ml, R&D systems) and isotype-matched IgG control, respectively, or purified clusterin (5 μg/ml) in 800 μl of serum-free DMEM. After incubation for 36 h, cells were fixed with 10% neutral buffered formalin, and stained with 0.1% crystal violet. Non-migrated cells were removed from the upper surface with a cotton swab and numbers of cells that had traversed the filters were counted.

3. Results

3.1. Clusterin upregulated the expressions of chemotactic cytokines in Raw264.7 macrophages

In previous study, we found that clusterin induces MMP-9 expression and activity in macrophages [12]. This observed led us to investigate whether clusterin affects the productions of other cytokines by macrophages. Because tumor-associated macrophages can stimulate metastasis by releasing cytokines that possibly create a favorable microenvironment for tumor progression, we used the mouse cytokine array to examine clusterin-stimulated Raw264.7 macrophages. Cells were stimulated with clusterin (1 μg/ml) for 24 h, and conditioned medium was collected and concentrated. As shown in Fig. 1A, cytokine array analysis showed that the secretions of granulocyte colony-stimulating factor (G-CSF), keratinocyte chemoattractant (KC), tumor necrosis factor-α (TNF-α, and monocyte chemotactic protein-1 (MCP-1) were significantly elevated by clusterin versus PBS-treated controls. It was also observed that macrophage inflammatory protein-1β (MIP-1β), interleukin-1 receptor antagonist (IL-1ra), and RANTES levels were moderately increased, whereas macrophage colony-stimulating factor (M-CSF) and interleukin-27 (IL-27) were slightly increased (data not shown). To determine whether these secretions were related to mRNA up-regulations, we isolated mRNAs from Raw264.7 cells cultured in the presence or absence of clusterin for 20 h. Subsequent RT-PCR showed that the mRNAs of the cytokines that were secreted after treatment with clusterin were up-regulated (Fig. 1B).

3.2. Clusterin stimulated TNF-α secretion by Raw264.7 macrophages

TNF-α is a potent proinflammatory cytokine produced predominantly by activated macrophages, and plays an important role in tumor progression via various mechanisms [13]. Based on our cytokine array findings, we evaluated whether clusterin dose-dependently induces TNF-α secretion. Spent media from Raw264.7 cells exposed to clusterin at concentrations of 0–4 μg/ml for 20 h were analyzed by Western blotting. As shown in Fig. 2A, clusterin was found to promote TNF-α secretion dose-dependently, peaking at a clusterin concentration of 4 μg/ml. In addition, Raw264.7 cells treated with 2 μg/ml of clusterin for the indicated times (0–20 h) also demonstrated time-dependent increases in TNF-α secretion (Fig. 2B). We then assessed whether pri-

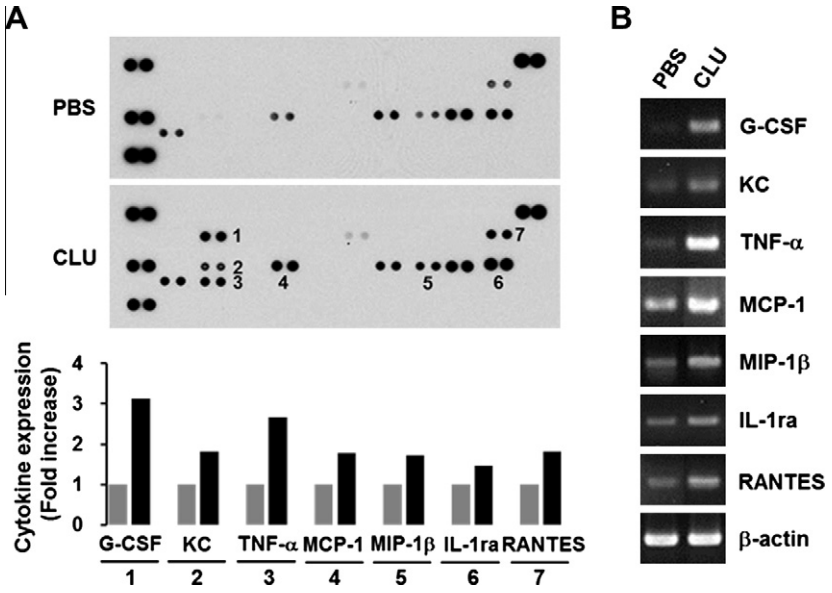

Fig. 1. Expression profiles of chemotactic cytokines in Raw264.7 macrophages after clusterin stimulation. (A) Mouse cytokine array. Raw264.7 cells were treated with clusterin (1 μg/ml) or PBS as vehicle for 24 h and then spent media were incubated with a mouse cytokine array as described in Section 2. Cytokines were spotted on the array panel in duplicate. Clusterin-induced cytokines were quantified by densitometric analysis using NIH ImageJ software (NIH, Bethesda, MA). (B) The expressions of mRNAs encoding cytokines induced by clusterin. RT-PCR was performed to confirm cytokine array data.

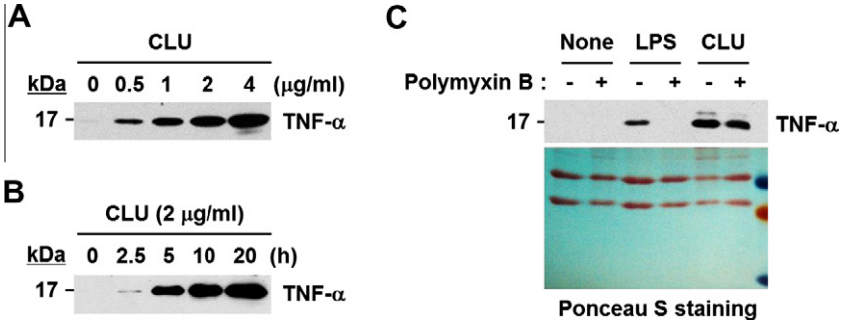

Fig. 2. Macrophage TNF-α secretion was induced by clusterin. (A) Dose–response analysis of TNF-α release with increasing concentration of clusterin. (B) Time-course analysis of TNF-α release in response to clusterin. Raw264.7 macrophages were treated with clusterin (0–4 μg/ml) for 20 h or treated with 2 μg/ml of clusterin for the indicated times. (C) Clusterin-triggered TNF-α secretion in primary mouse macrophages. Peritoneal macrophages from C57BL/6 mice were treated with LPS (100 ng/ml) or clusterin (2 μg/ml) for 24 h. Ponceau S-stained membranes were used as loading controls.

mary macrophages also release TNF-α in response to clusterin, and found that thioglycolate-elicited peritoneal macrophages from normal C57BL/6 mice also exhibited clusterin-induced TNF-α secretion (Fig. 2C, upper panel). To rule out the possibility that clusterin-induced TNF-α secretion was due to LPS contamination in our clusterin preparation, we added polymyxin B (a pharmacologic LPS antagonist) to cultures prior to clusterin stimulation. Although polymyxin B clearly diminished LPS-stimulated TNF-α secretion, it did not significantly influence clusterin-induced TNF-α secretion. These findings demonstrate the role played by clusterin in the promotion of macrophage TNF-α secretion. Ponceau S staining of the membrane was used as a protein loading control for soluble TNF-α in primary macrophage media (Fig. 2C, bottom panel).

3.3. The activations of the ERK, JNK and PI3K/Akt pathways were involved in clusterin-induced TNF-α secretion in Raw264.7 macrophages

The activations of mitogen-activated protein (MAP) kinases have been implicated in inflammatory response [14] and are well known to participate in TNF-α biosynthesis in macrophages [15]. Therefore, we first examined the activations of MAP kinases to identify the intracellular signaling molecules mediating the secretion of TNF-α by clusterin. Raw264.7 cells treated with clusterin (1 μg/ml) for different times showed rapid and sustained (up to 120 min) phosphorylations of ERK and JNK, but not of p38 (data not shown). Maximal activations of ERK and JNK occurred at 60 min after clusterin stimulation (Fig. 3A). Akt is another important regulator linked to the induction of TNF-α [16], and thus we investigated Akt activation in clusterin-treated Raw264.7 cells. As shown in Fig. 3A, the phosphorylation of Serine 473 of Akt (a hallmark of full Akt activation) appeared after 60 min of clusterin treatment. Total forms of ERK, JNK and Akt were used as internal standards to quantify their respective phosphorylated forms.

To determine the roles of the activations of ERK, JNK, and Akt in clusterin-induced TNF-α secretion, cells were pretreated with ERK inhibitor (PD98059), p38 inhibitor (SB203850), JNK inhibitor (SP600125), or PI3K inhibitor (LY294002), and then secreted TNF-α levels induced by clusterin were monitored. As shown in Fig. 3B, pretreatment with PD98059, SP600125, or LY294002, but not with SB203850 reduced clusterin-induced TNF-α production.

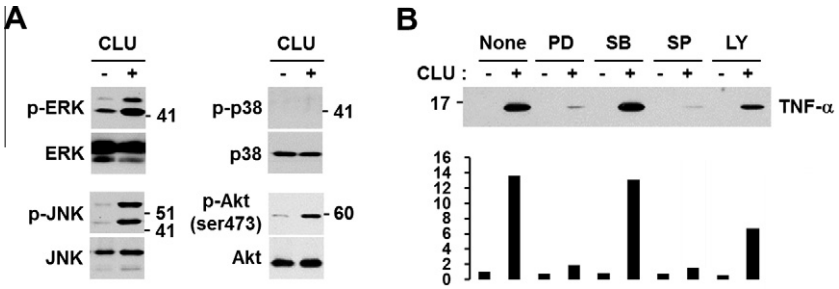

Fig. 3. Involvements of ERK, JNK, and PI3K/Akt in clusterin-induced TNF-α secretion. (A) The activations of ERK, JNK and Akt by clusterin. Raw264.7 cells were treated with clusterin (1 μg/ml) for 60 min and the phosphorylated (p) and total forms of ERK, p38, JNK, and Akt were evaluated by Western blotting. (B) Effect of PD (PD98059; ERK inhibitor), SB (SB203580; p38 inhibitor), SP (SP600125; JNK inhibitor), and LY (LY294002; PI3K inhibitor) on the clusterin-induced secretion of TNF-α. Cells were preincubated with each inhibitor prior to clusterin stimulation [PD (20 μM), SB (10 μM), SP (10 μM), LY (20 μM)]. Clusterin-induced TNF-α secretions in the presence of these pharmacological inhibitors were quantified by densitometric analysis using NIH ImageJ software.

These findings indicate that three pathways, namely, ERK, JNK, and PI3K/Akt, are involved in the clusterin-stimulated production of TNF-α in Raw264.7 macrophages.

3.4. Macrophage chemotaxis was enhanced by clusterin-induced TNF-α and directly by clusterin

TNF-α acts directly as a chemoattractant for macrophages or monocytes [5,17] or by enhancing the syntheses of chemokines like MCP-1 [18]. We examined whether the TNF-α secreted by Raw264.7 cells in clusterin-conditioned medium (CLU-CM) could enhance macrophage chemotactic activity. To address this, CLU-CM generated by clusterin treatment was filtered using an Amicon Ultra filter with 50 kDa cut off to remove clusterin and transferred to naïve Raw264.7 macrophages in a transwell filter assay system in the presence of normal goat (N-gt) IgG or anti-TNF-α neutralizing antibodies. As shown in Fig. 4A (left panel), CLU-CM (N-gt IgG) significantly increased the migration of Raw264.7 macrophages versus PBS-treated control Raw264.7 medium (PBS-CM). Furthermore, the CLU-CM induced migration of Raw264.7 macrophages was markedly reduced by preincubating CLU-CM with anti-TNF-α neutralizing antibodies. Chemotactic migration was analyzed by calculating the proportion of cells that had migrated to the lower side of the filter based on direct microscopic counts after staining with crystal violet. CLU-CM containing N-gt IgG showed 3.5-fold increase in cell migration versus PBS-CM, however, when neutralized with TNF-α antibodies, cell migration induced by CLU-CM was reduced 2.3-fold (right panel).

Tumor cells attract macrophages or monocytes by producing chemotactic agents, such as, TNF-α, MCP-1, M-CSF, and TGF-β, and by using as yet undefined mediators to create metastatic tumor microenvironments [19]. We hypothesized that clusterin overexpression by cancer cells per se might act as a chemoattractant for tumor-associated macrophages or blood monocytes and induce the secretions of several chemokines, including TNF-α, by macrophages. To address this, transwell filter assays were performed to quantify macrophage migration induced by clusterin. In a preliminary study, clusterin was found to be able to induce significant Raw264.7 macrophage migration in a concentration-dependent manner with peak activity at 5 μg/ml, and time course analysis also showed an increase in cell migration with time (data not shown). As shown in Fig. 4B, macrophage migration was significantly enhanced by 3.8-fold in clusterin-treated cells as compared with the spontaneous migration displayed by PBS-treated control cells. Taken together, these results show that clusterin-induced TNF-α is sufficient to stimulate macrophage migration, but that other chemokines induced by clusterin and clusterin itself also have the ability to evoke the chemotactic migration of macrophages.

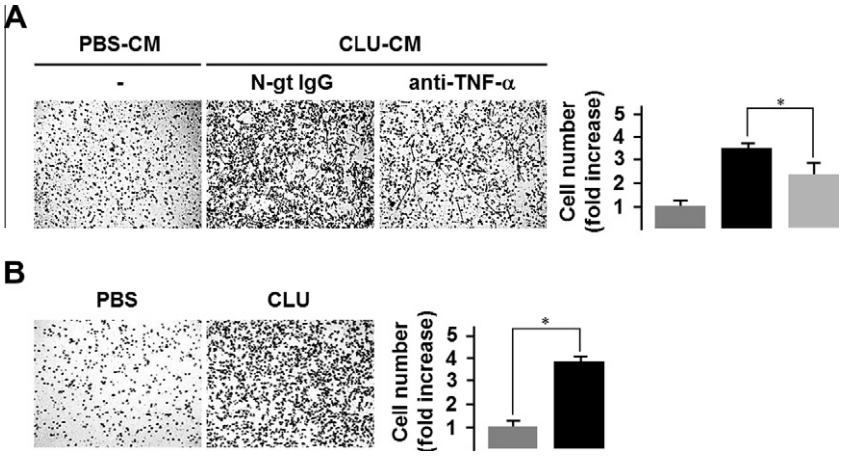

Fig. 4. Macrophage chemotaxis by clusterin-induced TNF-α or by clusterin. (A) The chemotactic effects of conditioned media (CM) from Raw264.7 macrophages exposed to PBS (PBS-CM) or clusterin (CLU-CM) containing normal goat IgG (N-gt IgG) or TNF-α neutralizing antibodies (anti-TNF-α). Raw264.7 cells were treated with clusterin for 24 h, the conditioned media were collected, and clusterin was removed using an Amicon Ultra filter with a 50 kDa cutoff. The resulting eluates were centrifuged and preincubated with N-gt IgG or anti-TNF-α before adding them to a lower transwell chamber as chemoattractants for naive Raw264.7 cells. (B) The chemotactic response of Raw264.7 macrophages to purified clusterin. Cells were treated with clusterin (5 μg/ml) in the lower chamber for 36 h. The results represent the means ± SEM of three separate experiments. $^{*}p < 0.05$ compared to N-gt IgG- or PBS-treated control.

4. Discussion

The role played by clusterin in cancer biology has not been clearly elucidated. Clusterin functions primarily through two isoforms, an anti-apoptotic secreted form and a pro-apoptotic intracellular form. Several experimental reports have claimed that clusterin (in its secreted form) plays an important role in tumor growth and metastatic progression [11,20,21]. Furthermore, it is believed that the pro-tumor effect of clusterin is due to its anti-apoptotic chaperone activity, leading to tumor cell survival via the blockage of apoptotic signaling. In the present study, we offer a novel mechanism for clusterin-mediated tumor growth via the regulation of macrophages, and report that clusterin promotes the mRNA expressions and secretions of a number of chemokines, including TNF-α, by macrophages. In addition, we demonstrate that ERK, JNK, and PI3K/Akt signaling pathways participate in the clusterin-induced secretion of TNF-α, and that this contributes to the macrophage chemotaxis triggered by clusterin. These findings support the hypothesis that clusterin derived from metastatic-prone tumor cells, serves as a tumor-promoting factor by inducing the secretion of TNF-α and of other chemokines responsible for metastasis from surrounding macrophages.

Most solid tumors *in vivo* communicate reciprocally with stroma, including macrophages, to promote metastatic spread by altering local and distant microenvironments via the secretions of specific cytokines. Leukocyte infiltration, that is, the recruitment of macrophages or blood monocytes to tumor site, is critical for metastatic progression [22], and is mediated by various chemotactic cytokines from tumor cells or resident macrophages via paracrine interactions. The present study shows that clusterin is able to stimulate chemokine release from Raw264.7 macrophages including TNF-α and promote macrophage migration (Fig. 4). However, our TNF-α neutralization experiment showed incomplete inhibition of cell migration induced by clusterin, and thus suggested the presence of other chemotactic factor(s) in clusterin-treated Raw264.7 media. Indeed, as shown in Fig. 1, clusterin triggered the expressions and secretions of MCP-1, MIP-1β, RANTES, KC, and M-CSF, which are all known chemoattractants of macrophages and monocytes [5,23–25], thus we cannot rule out the involvements of these chemokines in clusterin-enhanced macrophage chemotaxis, despite the fact that TNF-α is a master regulator of the productions of proinflammatory cytokines, such as, MCP-1 and KC.

Moreover, we found that not only CLU-CM (chemokines secreted from macrophages by clusterin treatment), but also clusterin itself enhanced the migration of macrophages. The effects of clusterin on cell migration were previously reported to be either stimulatory on cancer cells [26] or inhibitory on vascular smooth muscle cells [27]. It is unclear whether this opposite effect was due to either the cell types or the clusterin isoforms (intracellular versus secretory forms), since the overexpression was achieved by introducing clusterin gene into cells in those studies. In accordance with our study using purified clusterin, Lenferink et al., showed that clusterin depletion by neutralizing antibodies could inhibit TGF-β induced motility of cancer cells, providing evidence that a secretory form of clusterin promote cell migration [28]. Interestingly, we found that the purified clusterin enhanced the migration of macrophages in a positive correlation with time of storage at 4 °C. Thus, it was speculated that the degraded form of clusterin may be more effective for chemotactic migration of macrophages. To test this speculation, we allowed the purified clusterin to degrade for three months at 4 °C, whereas the intact protein was usually stored at −20 °C, and then we monitored the chemotactic migration of Raw264.7 macrophages. After 3 months of storage at 4 °C, only two faint bands of degraded clusterin at 45–50 kDa (dCLU) were visible, but the ~70 kDa intact protein (iCLU) was not observed by Coomassie blue staining or western blotting (Supplementary Fig. S1A). Although its sequences and structures have not been determined, the degraded clusterin had greater chemotactic activity for macrophages than the intact protein (Supplementary Fig. S1B). On the other hand, the effect of clusterin on the MMP-9 activity was found to be opposite. When the intact form of clusterin (lane 3) was treated to Raw264.7 macrophages, zymography showed a greater MMP-9 activity than the degraded form (Supplementary Fig. S2, upper panel). Partial degradation of clusterin (lane 2) showed a reduced MMP-9 gelatinase activity, and furthermore, completely degraded clusterin with no bands by western blotting had no effect on MMP-9 activity (lane 1). This is consistent with our previous result that the intact secretory form of clusterin promoted MMP-9 activation, while both glycosylation-deficient clusterin by *N*-glycosidase and a non-glycosylated recombinant clusterin obtained from E. coli were unable to stimulate MMP-9 in macrophages [12]. These data describe a differential effect of clusterin on chemotactic migration and MMP-9 activity of macrophages, depending on the extent of degradation. It is not surprising since certain plasma proteins, such as, fibronectin, fibrinogen, and albumin, play important physiological roles in their degraded and intact forms *in vivo* [29–31]. Thus, we report for the first time an enhanced chemotactic activity by the degraded form of clusterin, although its sequence and structure remain to be elucidated.

In conclusion, our results demonstrate that clusterin can stimulate various chemotactic cytokines, including TNF-α and play a critical role in macrophage chemotaxis. Furthermore, the ERK, JNK, and PI3K/Akt pathways are involved in clusterin-induced TNF-α expression and secretion. In addition, the partial degraded clusterin has greater chemotactic activity for macrophages. Collectively, these findings underscore that clusterin acts as an inducer of TNF-α expression in macrophages and of macrophage chemotactic migration, and suggest that these relations are responsible for the tumor-promoting effect of clusterin.

Acknowledgment

This work was supported by the Korea Science and Engineering Foundation (KOSEF) funded by the Korea government (MEST) (Grant No. 2011-002-8200).

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.2012.04.162.

References

[1] C.E. Green, T. Liu, V. Montel, G. Hsiao, R.D. Lester, S. Subramaniam, S.L. Gonias, R.L. Klemke, Chemoattractant signaling between tumor cells and macrophages regulates cancer cell migration, metastasis and neovascularization, PLoS One 4 (2009) e6713.
[2] G.J. van der Bij, S.J. Oosterling, S. Meijer, R.H. Beelen, M. van Egmond, The role of macrophages in tumor development, Cell Oncol 27 (2005) 203–213.
[3] J.A. Joyce, J.W. Pollard, Microenvironmental regulation of metastasis, Nat. Rev. Cancer 9 (2009) 239–252.
[4] C.E. Lewis, J.W. Pollard, Distinct role of macrophages in different tumor microenvironments, Cancer Res. 66 (2006) 605–612.
[5] A.C. Issekutz, T.B. Issekutz, Quantitation and kinetics of blood monocyte migration to acute inflammatory reactions, and IL-1 alpha, tumor necrosis factor-alpha, and IFN-gamma, J. Immunol. 151 (1993) 2105–2115.
[6] D.E. Jenne, J. Tschopp, Clusterin: the intriguing guises of a widely expressed glycoprotein, Trends Biochem. Sci. 17 (1992) 154–159.
[7] M. Gleave, K.N. Chi, Knock-down of the cytoprotective gene, clusterin, to enhance hormone and chemosensitivity in prostate and other cancers, Ann. NY Acad. Sci. 1058 (2005) 1–15.
[8] M. Redondo, T. Tellez, M.J. Roldan, The role of clusterin (CLU) in malignant

transformation and drug resistance in breast carcinomas, Adv. Cancer Res. 105 (2009) 21–43.
[9] L.V. July, E. Beraldi, A. So, L. Fazli, K. Evans, J.C. English, M.E. Gleave, Nucleotide-based therapies targeting clusterin chemosensitize human lung adenocarcinoma cells both in vitro and in vivo, Mol. Cancer Ther. 3 (2004) 223–232.
[10] J.A. Sensibar, D.M. Sutkowski, A. Raffo, R. Buttyan, M.D. Griswold, S.R. Sylvester, J.M. Kozlowski, C. Lee, Prevention of cell death induced by tumor necrosis factor alpha in LNCaP cells by overexpression of sulfated glycoprotein-2 (Clusterin), Cancer Res. 55 (1995) 2431–2437.
[11] L. Flanagan, L. Whyte, N. Chatterjee, M. Tenniswood, Effects of clusterin over-expression on metastatic progression and therapy in breast cancer, BMC Cancer 10 (2010) 107.
[12] 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.
[13] P. Szlosarek, K.A. Charles, F.R. Balkwill, Tumour necrosis factor-alpha as a tumour promoter, Eur. J. Cancer 42 (2006) 745–750.
[14] J.S. Schorey, A.M. Cooper, Macrophage signalling upon mycobacterial infection: the MAP kinases lead the way, Cell Microbiol. 5 (2003) 133–142.
[15] V. Debbas, R.J. Arai, S. Ferderbar, F. Schindler, A. Stern, H.P. Monteiro, Regulation of p21Waf1 expression and TNFalpha biosynthesis by glutathione modulators in PMA induced-THP1 differentiation: involvement of JNK and ERK pathways, Biochem. Biophys. Res. Commun. 363 (2007) 965–970.
[16] D.L. Williams, T. Ozment-Skelton, C. Li, Modulation of the phosphoinositide 3-kinase signaling pathway alters host response to sepsis, inflammation, and ischemia/reperfusion injury, Shock 25 (2006) 432–439.
[17] T.B. Issekutz, In vivo blood monocyte migration to acute inflammatory reactions, IL-1 alpha, TNF-alpha, IFN-gamma, and C5a utilizes LFA-1, Mac-1, and VLA-4. The relative importance of each integrin, J. Immunol. 154 (1995) 6533–6540.
[18] L.K. Sun, T. Reding, M. Bain, M. Heikenwalder, D. Bimmler, R. Graf, Prostaglandin E2 modulates TNF-alpha-induced MCP-1 synthesis in pancreatic acinar cells in a PKA-dependent manner, Am. J. Physiol. Gastrointest Liver Physiol. 293 (2007) G1196–1204.
[19] B. al-Sarireh, O. Eremin, Tumour-associated macrophages (TAMS): disordered function, immune suppression and progressive tumour growth, J. R. Coll. Surg. Edinb. 45 (2000) 1–16.
[20] J. Bi, A.L. Guo, Y.R. Lai, B. Li, J.M. Zhong, H.Q. Wu, Z. Xie, Y.L. He, Z.L. Lv, S.H. Lau, Q. Wang, X.H. Huang, L.J. Zhang, J.M. Wen, X.Y. Guan, Overexpression of clusterin correlates with tumor progression, metastasis in gastric cancer: a study on tissue microarrays, Neoplasma 57 (2010) 191–197.
[21] L. Wei, T. Xue, J. Wang, B. Chen, Y. Lei, Y. Huang, H. Wang, X. Xin, Roles of clusterin in progression, chemoresistance and metastasis of human ovarian cancer, Int. J. Cancer 125 (2009) 791–806.
[22] Y.G. Man, Aberrant leukocyte infiltration: a direct trigger for breast tumor invasion and metastasis, Int. J. Biol. Sci. 6 (2010) 129–132.
[23] J.G. Neels, L. Badeanlou, K.D. Hester, F. Samad, Keratinocyte-derived chemokine in obesity: expression, regulation, and role in adipose macrophage infiltration and glucose homeostasis, J. Biol. Chem. 284 (2009) 20692–20698.
[24] M. Keophiphath, C. Rouault, A. Divoux, K. Clement, D. Lacasa, CCL5 promotes macrophage recruitment and survival in human adipose tissue, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 39–45.
[25] 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.
[26] S.H. Lau, J.S. Sham, D. Xie, C.H. Tzang, D. Tang, N. Ma, L. Hu, Y. Wang, J.M. Wen, G. Xiao, W.M. Zhang, G.K. Lau, M. Yang, X.Y. Guan, Clusterin plays an important role in hepatocellular carcinoma metastasis, Oncogene 25 (2006) 1242–1250.
[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] 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.
[29] E. Franco, J. Vazquez-Prado, I. Meza, Fibronectin-derived fragments as inducers of adhesion and chemotaxis of Entamoeba histolytica trophozoites, J. Infect. Dis. 176 (1997) 1597–1602.
[30] J. Trial, R.D. Rossen, J. Rubio, A.A. Knowlton, Inflammation and ischemia: macrophages activated by fibronectin fragments enhance the survival of injured cardiac myocytes, Exp. Biol. Med. (Maywood) 229 (2004) 538–545.
[31] M. Zarebski, W. Sobaniec, J. Moniuszko-Jakoniuk, J. Mackowiak, The effect of leukocyte-induced degradation products of albumin on the analgesic action of morphine, Acta Neurobiol. Exp. (Wars) 36 (1976) 373–380.