대 한 체 질 인 류 학 회 지
Korean J Phys Anthropol
20(3): 225 ~ 233, 2007

CD98 Activation Increases the Invasion of Human Breast Carcinoma MCF-7 Cells

Sun-Mi Kim, Jae-Myoung Kim, Joon-Seong Jeon[1], Zoon-Seog Ahn, Dae-Young Kim, Jae-Sung Suh, Kyoung-Han Park, Jung-Hyun Park, Dae-Joong Kim, Jang-Hee Hahn

Department of Anatomy and Cell Biology, College of Medicine, Kangwon National University
[1]Department of Urology, College of Medicine, Inje University

Abstract : CD98, a disulfide-linked 125-kDa heterodimeric type II transmembrane glycoprotein, regulates the func-tions of β_1 integrin, suggesting that it may play a role in tumor cell invasion. In this study, the effects of CD98 signaling on the adhesion and invasion of tumor cells were investigated.

The expression of CD98 on MCF-7 human breast carcinoma cells was confirmed by immunohistochemistry. The effects of CD98 activation on the adhesion to extracellular matrix (ECM) and invasion of MCF-7 cells were determined by adhesion assay and cell invasion assay. Dominant negative forms of focal adhesion kinase (FAK) were transiently transfected into MCF-7 cells using liposome reagents.

CD98 stimulation increased the adhesion of MCF-7 cells to fibronectin, laminin and collagen IV. Activation of CD98 augmented the invasion rate of MCF-7 cells through ECM. EDTA or a function-blocking anti-β_1 integrin mAb suppressed the effect of CD98 on invasiveness. Inhibition of phosphorylation of FAK by PP2, an inhibitor of Src family kinase, reduced CD98-induced invasion of MCF-7 cells. This result was confirmed by over-expression of dominant negative forms of FAK. In addition, cytochalasin D or phalloidin inhibited CD98-mediated induction of tumor cell invasion. Inhibitory effects of PP2, cytochalasin D or phalloidin on CD98-stimulated invasion of MCF-7 cells were diminished by pretreatment of cells with Mn^{++}, which is shown to induce conformational change of β_1 intgerin.

These results provide the first evidence that CD98 activation increases tumor cell invasion by activating β_1 integrin affinity, and that FAK phosphorylation and subsequent cytoskeletal reorganization may be essential for CD98-mediated regulation of cell motility.

Key words : CD98, β_1 integrin, Tumor cell invasion, Cell-ECM adhesion, FAK, Actin, Cytoskeleton

Introduction

The human CD98 protein, a disulfide-linked 125-kDa heterodimeric type II transmembrane glycoprotein, is composed of a glycosylated 85-kDa heavy chain (designated CD98) and a nonglycosylated 40-kDa light chain. CD98 is broadly expressed in various normal cells and many tumor cell types (Bellone et al. 1989, Dixon et al. 1990). It has been shown that CD98 may be involved in regulation of integrin function,

*This work was supported by Vascular System Research Center grant from the Korea Science & Engineering Foundation
Correspondence to : Jang-Hee Hahn (Department of Anatomy and Cell Biology, College of Medicine, Kangwon National University)
E-mail : jhahn@kangwon.ac.kr

amino acid transport, cell fusion, and proliferation (Deves and Boyd 2000). CD98 stimulation enhanced β_1 integrin-mediated adhesion of the small cell lung-cancer cell line (SCLC) H345 and breast cancer cells to fibronectin and laminin by converting β_1 integrins into a high-affinity state (Fenczik et al. 1997, Chandrasekaran et al. 1999). It was previously reported that cross-linking CD98 induced the clustering of β_1 integrins and integrin-like signaling (Rintoul et al. 2002). This convergent signaling mechanism between integrins and CD98 is supported by the observations that CD98 is associated with integrins (Zent et al. 2000, Fenczik et al. 2001). It has been well known that regulation of integrin activity is required for the migratory phenotype of tumor cells; it controls not only cell-ECM adhesion, but cytoskeletal dynamics for the motile machinery (Friedl et al. 2004). Taken together, these results suggest that CD98 signaling is mediated by β_1 integrin, and may be involved in the regulation of tumor cell invasion.

In this study, we investigate whether CD98 signaling regulates the invasion rate of MCF-7 human breast carcinoma cells, and if so, whether the effect of CD98 stimulation on cell invasion is mediated by FAK phosphorylation and the cytoskeleton reorganization. Our data demonstrate that CD98 signaling may regulate invasion of human breast carcinoma cells mainly by activating β_1 integrin affinity and that FAK and actin cytoskeleton are in-volved in this process.

Material and Methods

1. Cell and reagents

MCF-7 human breast carcinoma cell line was obtained from Korean Cell Line Bank (Seoul, Korea). Culture reagents were purchased from Invitrogen (Carlsbad, CA, USA). Anti-human CD98 mAb (UM7F8) was purchased from BD Biosciences (San Jose, CA, USA). Purified or R-PE conjugated goat anti-mouse IgG was from Dinona (Seoul, Korea). Blocking anti-β_1 integrin monoclonal antibody (JB1A) was from Chemicon International, Inc. (Temecula, CA, USA). PP2 was from Biomol Research Laboratories Inc. (Plymouth Meeting, PA, USA). Cytochalasin D and phalloidin were from Tocris Bioscience (Northpoint, Fourthway, Avonmouth, UK). Fibronectin, laminin, collagen type IV, poly-L-lysine, phalloidin-FITC and mouse IgG were purchased from Sigma-Aldrich (St. Louis, MO, USA). Liposome Gene-SHUTTLE-40 was from Qbiogene (Irvine, CA, USA).

2. Cell culture

MCF-7 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, 100 μg/mL streptomycin and 100 mM sodium pyrubate, 5 mg/mL insulin. Cell cultures were maintained and incubated in 5% CO_2 at 37˚C.

3. Immunohistochemistry

To determine the expression levels of CD98 on the surface of MCF-7 cells, cells were harvested with 200 μL of serum-free medium (SFM), fixed with 2% paraformaldehyde in PBS for 30 min at room temperature. After washing with PBS, they were permeabilized in 0.1% Triton x-100. Permiabilized cells were incubated with 1 μg of anti-human CD98 mAb and phalloidin-FITC for 1 h at room temperature. After washing with 1 mL of SFM twice, they were resuspended in 200 μL of SFM, containing 1 μg of R-PE-conjugated anti-mouse IgG antibody and incubated for 1 h at room temperature. Finally, samples were washed, mounted onto slides, embedded with mounting medium (DakoCytomation, Carpinteria, CA, USA), and analyzed under a confocal microscope (Leica TCS SP; Leica, Deerfield, IL, USA). Images were processed using the Adobe Photoshop software.

4. Transient transfection with FAK mutants

pcDNA3 vector (Invitrogen, Calsbard, CA, USA) encoding FAK Y397F mutant or FAK related non-kinase (FRNK) was kindly provided by Dr. Soo-Chul Park (Sookmyung Women's University). MCF-7 cells grown to 70～80% confluence were transiently transfected with the expression vector pcDNA3-FAK Y397F or pCDNA3-FRNK by using Gene SHUTTLE-40. pcDNA3 vector only was also transfected as mock vector. Each transfection mix also contained 1 μg of a β-galactosidase expression plasmid. After incubation overnight, adhesion rates of transfected cells were calculated by normalizing invasion rate against β-galactosidase activity.

5. Adhesion assay

Laminin, fibronectin, collagen type IV and poly-L-lysine were used as substrates in serum-free adhesion assays. Nonspecific binding sites were blocked with 1% BSA in PBS for 1 h at room temperature. 2 μg of fibronectin, laminin, collagen type IV or poly-L-lysine in PBS was coated on 96-well microtitre plates overnight at 4˚C. 5×10^4 MCF-7 cells in the 100 μL of serum free medium were allowed to adhere to fibronectin-, laminin-, collagen type IV-, PLL-coated 96-well plates, alone or after incubation with mouse IgG (3 μg) and goat anti-mouse IgG (30 μg), or anti-CD98 mAb (3 μg) and goat anti-mouse IgG (30 μg) for 1 h. After washing with PBS three times, cell attachment was determined by counting the number of attached cells. For adhesion assay with β_1 integrin-blocking antibody treatment, 5×10^4 MCF-7 cells in the 100 μL of SFM were incubated with anti-CD98 mAb (3 μg) in the presence or absence of β_1 integrin-blocking antibody (3 μg) and then plated on FN-coated plates. Adhesion rates were determined as described above.

6. Cell invasion assay

Polycarbonate filters (8-μm pore size) were coated with Matrigel and placed in a modified Boyden chamber. Boyden chambers were filled with 600 μL of NIH3T3 cell cultured-DMEM medium conditioned media, 0.005% vitamin C, 0.1% BSA as a source of chemoattractants in the lower compartment. Then, 100 μL of MCF-7 cell suspension (1×10^6 cells/mL) were placed in the upper compartment of the chamber. The plates were incubated at 37˚C in 5% CO_2 in air saturated with H_2O for 72 h. The cells on the lower surface of the filter were stained and counted every 24 h. Each assay was carried out in triplicate.

7. Statistical analysis

The data are expressed as the average of the mean values obtained ± SE. Statistical significance was determined by the Student's *t* test with the statistical software GraphPad Prism (version 4.0).

Results

1. Cross-linking CD98 enhances integrin-mediated cell adhesion

CD98 expression on the surface of MCF-7 cell lines in vitro was examined by using an indirect immunofluorescence technique with the anti-CD98 mAb UM7F8. Figure 1A shows that CD98 is highly expressed on the surface of MCF-7 cells. Consistent with the previous report (Kolesnikova et al. 2001), cross-linking CD98 led to formation of CD98 clusters. To investigate whether CD98 activation leads to any change in an adhesive phenotype of MCF-7 cells, we treated MCF-7 cells with anti-CD98 mAb UM7F8 and secondary Ab and then let them adhere to fibronectin, laminin, collagen IV or poly-L-lysine coated plates. Cross-linking CD98 led to up-regulation of cell adhesion to fibronectin, laminin and collagen IV (Fig. 1B). In contrast, adhesion to poly-L-lysine, which does not engage integrins, was not affected by CD98 activation. In order to investigate whether the stimulatory effect of

CD98 on cell-ECM adhesion is mediated by β_1 integrin activation, we inhibited the function of β_1 integrin with a blocking monoclonal antibody JB1A and then measured adhesiveness. Increased adhesion rate of CD98-treated cells was diminished by β_1 integrin-blocking antibody treatment (Fig. 1C). Consistent with the previous results showing that β_1 integrin affinity for ECM ligands was up-regulated by the activated CD98 (Fenczik et al. 1997, Chandrasekaran et al. 1999), these results indicate that CD98 activation enhances adhesion rate of MCF-7 cells to ECM by activating β_1 integrins.

2. CD98 signaling increases cell invasion by inducing conformational change of β_1 integrin

To investigate whether CD98 activation leads to any change in tumor cell invasion, MCF-7 cells were treated with anti-CD98 mAb or with mouse IgG as control and then let them invade Matrigel in the absence of serum. Cross-linking CD98 increased regulation of cell invasion through Matrigel compared with control (Fig. 2A and B). When MCF-7 cells were incubated in the presence of increasing concentrations

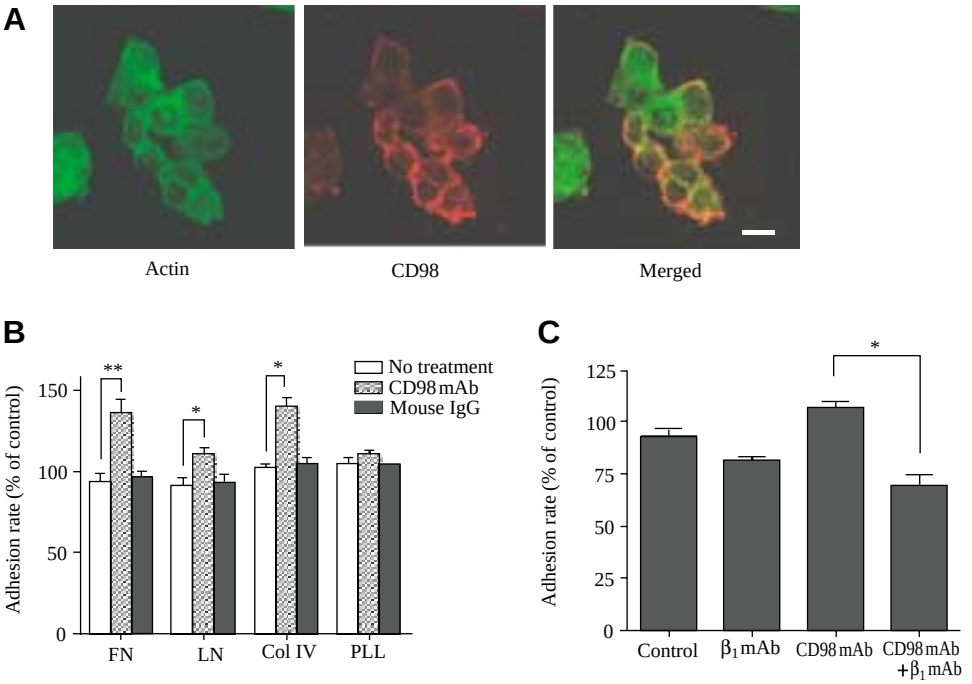

Fig. 1. Engagement of CD98 increases the adhesion rate of MCF-7 cells to ECM proteins by activating β_1 integrins. A. MCF-7 cells were incubated with anti-CD98 mAb and phalloidin-FITC, followed by R-PE-conjugated goat anti-mouse IgG. Confocal microscopy was performed to visualize CD98 expression and actin cytoskeleton in MCF-7 cells. Scale bar, 100 μm. Original magnification, ×100. B. MCF-7 cells were untreated (empty bars), incubated with anti-CD98 mAb (shaded bars), or mouse IgG (filled bars), and then plated on fibronectin-(FN), laminin-(LN), collagen IV-(Col IV) or poly-L-lysine (PLL)-coated dishes. After washing two times, adhesion rates were determined by counting the number of control cells or antibody-treated cells binging to fibronectin, laminin, collagen IV or poly-L-lysine. Data represent the mean±SE of triplicate determinations (*$P<0.05$, **$P<0.01$). C. MCF-7 cells were incubated with anti-CD98 mAb in the absence or presence of a function blocking anti-β_1 integrin mAb, and then plated on fibronectin-coated dishes. After washing two times, adhesion rates were determined as described above (*$P<0.001$).

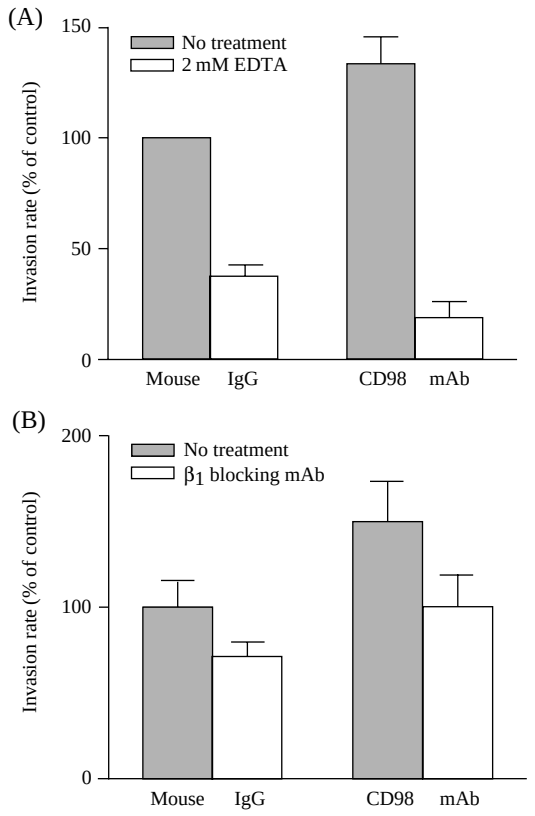

Fig. 2. CD98 engagement increases the invasion rate of MCF-7 cells by activating β_1 integrins. MCF-7 cells were incubated with mouse IgG (3 μg) and secondary Ab (30 μg) as control, or anti-CD98 mAb (3 μg) and secondary Ab (30 μg) in the presence (empty bars) or absence (filled bars) of 2 mM EDTA (A), or 4 μg of anti-β_1 integrin blocking mAb (B). The invasion of cells across Matrigel membranes was determined every 24 h as described under "Materials and Methods". Invasion rate was determined by counting the total number of control cells or anti-CD98 mAb-treated cells migrating through Matrigel for 72 h and dividing the number of anti-CD98 mAb-treated cells by that of control cells. Data are presented as the mean ± SE of four independent experiments.

of anti-CD98 mAb, a dose-dependent increase in cell invasion was seen (data not shown).

Next, we examined whether CD98-induced conformational changes in β_1 integrins play a role in increased cell invasiveness by CD98 activation. We treated MCF-7 cells with EDTA (2 mM), a Mn^{++} chelator, or β_1 integrin-blocking antibody, which converts integrins in an inactive conformation, before stimulating cells with CD98 mAb. Treatment of cells with EDTA drastically suppressed the effect of CD98 signaling on invasiveness (Fig. 2A). In addition, addition of β_1 integrin blocking antibody reversed the stimulatory effects of CD98 (Fig. 2B). These results suggest that CD98 activation may stimulate cell invasion by converting β_1 integrin into an active conformation.

3. CD98 signaling regulates cell invasion in the FAK-dependent pathway

Previously, it was shown that cross-linking or overexpression of CD98 causes FAK phosphorylation (Rintoul et al. 2002, Cai et al. 2005). To investigate the effects of FAK phosphorylation on the CD98-mediated regulation of cell invasion, we treated MCF-7 cells with anti-CD98 mAb in the absence and presece of PP2 (0.2 μM), which prevents phosphorylation of FAK by inhibiting Src kinase activity. PP2 treatment inhibited the effect of CD98 signaling on cell invasion, suggesting that the increased FAK phosphorylation is required for CD98-induced cell invasion (Fig. 3A). However, simultaneous treatment of MCF-7 cells with PP2 and Mn^{++} (0.5 μM) restored the effect of CD98 signaling on cell invasion. To further confirm involvement of FAK in CD98-mediated cell invasion, we examined the effect of overexpression of dominant-negative forms of FAK on CD98 signaling in cell invasion. As shown in Fig. 3B, the overexpression of dominant-negative forms of FAK significantly decreased the effect of CD98 on cell invasion. Addition of Mn^{++} (0.5 μM) to these transient transfected cells restored partially the effect of CD98 signaling on cell invasion.

4. CD98 regulates cell invasion through reorganization of cytoskeleton

To investigate whether actin reorganization plays a role in the intracellular signaling pathways responsible

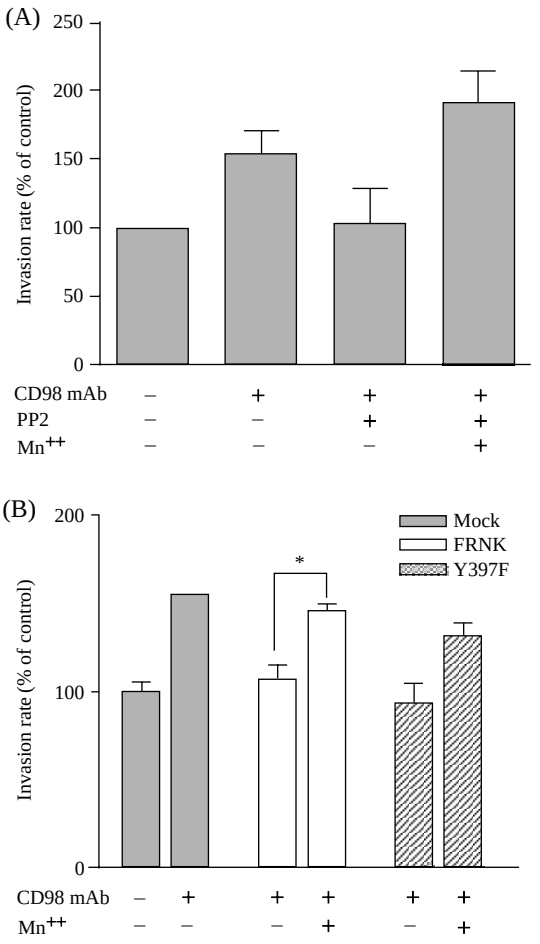

Fig. 3. Inhibition of FAK blocks CD98-induced cell invasion. (A) MCF-7 cells were untreated as control, or treated with anti -CD98 mAb in the presence or absence of PP2 (0.2 μM) for 1 h. In addition, whether addition of 0.5 μM Mn^{++} interferes with the effect of PP2 on CD98-induced cell invasion was determined. (B) MCF-7 cells were transiently transfected with pcDNA3 as mock vector (filled bars), pcDNA 3-FRNK (empty bars) or pcDNA3-Y397F (shaded bars) plasmid DNA, and then were incubated with anti-CD98 mAb in the presence or absence of 0.5 μM Mn^{++} for 1 h. The effects of inhibition of FAK on cell invasion rate were determined as described in "Material and Me-thod". Results are expressed as mean ± SE of three inde-pendent experiments (*$P < 0.001$).

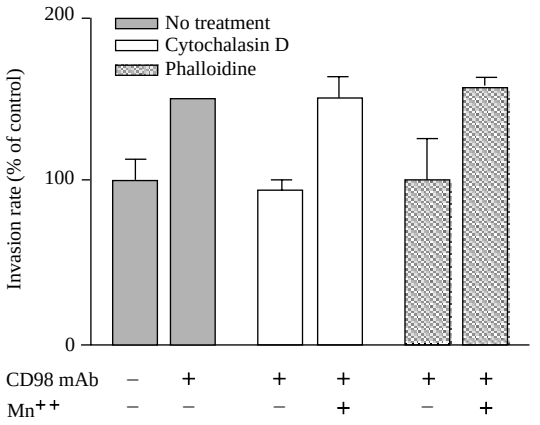

Fig. 4. Inhibition of cytoskeletal reorganization suppresses the stimulatory effect of CD98 on invasiveness. MCF-7 cells were untreated, or treated with 4 μM cytochalasin D or 10 μM phalloidin in the presence of anti-CD98 mAb for 1 h. The effect of cytochalasin D or phalloidin treatment on cell invasion rate was determined as described in "Material and Method". In addition, whether addition of 0.5 μM Mn^{++} interferes with the effect of cytochalasin D or phalloidin on CD98-induced cell invasion was determined by the same way. Results are expressed as mean ± SE of three independent experiments.

for the CD98 functions in cell invasion, we pretreated MCF-7 cells with pharmacological signaling inhibitors, cytochalasin D for actin filament polymerization or phalloidin for actin reorganization. Exposure of MCF-7 cells to 4 μM of cytochalasin D completely blocked CD98-mediated induction of cell invasion (Fig. 4). Pretreatment of MCF-7 cells with 10 μM of phalloidin also resulted in inhibition of CD98 effects on cell invasion. Interestingly, these inhibitory effects of cytochalasin D and phalloidin were restored by Mn^{++}-mediated activation of β_1 integrin affinity. Thus, reorganization of actin cytoskeleton is required for CD98-mediated induction of cell invasion.

Discussion

In this study, we have examined the effect of CD98 activation on the invasiveness of MCF-7 human breast carcinoma cells. This study has shown that, first, cross

-linking CD98 induced cell invasion through Matrigel by increasing β_1 integrin affinity. Second, suppression of phosphorylation of FAK by PP2 or overexpression of dominant negative mutants of FAK inhibited the CD98 signal-mediated induction of invasion of MCF-7 cells. Finally, cytochalasin D or phalloidin that inhibits actin reorganization prevented CD98 signaling from increasing cell invasion.

Previous studies have demonstrated that CD98 plays a role in β_1 integrin regulation (Fenczik et al. 1997). Several mechanisms could account for this role including alterations in expression levels on the cell surface, modulation of receptor avidity by lateral diffusion of heterodimers to form clusters, or modulation of receptor affinity by conformational changes in the heterodimer. Our previous study showed that cell surface expression of β_1 integrin was enhanced by engagement of CD98 with anti-CD98 mAb, suggesting that the CD98 enhances the activity of β_1 integrin by augmenting cell surface expression of active β_1 integrin quantitatively (Ahn 2003). In addition, cross-linking CD98 induces clustering of β_1 integrin on MCF-7 cells (Jeon 2004). Clustering of integrins has been known to be an important component that governs the overall strength of cellular adhesiveness. On the other hand, this study shows CD98-induced cell adhesion was inhibited by chelating Mn^{++} or treatment with β_1 integrin blocking antibody. These results suggest that conformational changes of β_1 integrin may play a key role in CD98-induced cell adhesion. Thus, some combination of mechanisms described above may be involved in CD98-mediated regulation of β_1 integrin activity, although it is difficult to determine the relative contributions of each (Hato et al. 1998).

CD98-induced cell invasion was significantly reduced by the pretreatment with PP2, an inhibitor of Src family kinase. These results were consistent with previous reports (Rintoul 2002, Cai 2005) and confirmed by over-expression of dominant negative mutants of FAK in this study. Previous studies showed that cross-linking CD98 increased phosphorylation of FAK dependent of β_1 integrin-mediated signaling pathway. Since CD98 specifically associates with β_1 integrins, stimulation of CD98 leads to activation of β_1 integrin. One of the initial events triggered by the stimulation of β_1 integrin is the association of its cytoplasmic domain with FAK followed by tyrosine phosphorylation and activation of FAK. Functional molecular interaction has been shown between the β_1 integrin cytoplasmic domain and the FAK C-terminal domain. FAK is involved in the regulation of cell adhesion or cytoskeletal dynamics and affects cell motility (Parsons et al. 2000, Mitra et al. 2005). In this study, we investigated whether CD98-dependent enhancement of cell invasion requires intact actin integrity and involves cytoskeletal reorganization. The possible involvement of actin cytoskeleton was tested by pretreating MCF-7 cells with cytochalasin D or phalloidin before CD98 cross-linking. Pretreatment with cytochalasin D or phalloidin clearly prevented CD98-stimulated invasion. These results suggest that an intact actin cytoskeleton may be required for CD98 to stimulate invasion. Destabilization of actin cytoskeleton might inhibit cells from migrating through ECM. Interestingly, conformational change of β_1 integrin with $MnCl_2$ treatment restored the inhibitory effect of PP2 or dominant negative forms of FAK on CD98-stimulated cell invasion. Likewise, it restored the invasion rate of cytochalasin D or phalloidin-treated cells. Mn^{++}-mediated activation of β_1 integrin might induce phosphorylation of FAK and cytoskeletal reorganization, which will subsequently stabilize adhesion complex and restore cell invasiveness.

In summary, this study provides the first evidence that CD98 activation increases tumor cell invasion by activating β_1 integrin affinity and that phosphorylated FAK and the integrity of the actin cytoskeleton may be essential for CD98-mediated regulation of cell motility.

References

Ahn ZS : Studies on control of invasiveness of human breast carcinoma cell line MCF-7 by cell surface molecule CD98. Graduate School Kangwon National University 2003. (in Korean)

Bellone G, Alloatti G, Levi R, Geuna M, Tetta C, Peruzzi L, Letarte M, Malavasi F : Identification of a new epitope of the 4F2/44D7 molecular complex present on sarcolemma and isolated cardiac fibers. Eur J Immunol 19: 1-8, 1989.

Cai S, Bulus N, Fonseca-Siesser PM, Chen D, Hanks SK, Pozzi A, Zent R : CD98 modulates integrin beta1 function in polarized epithelial cells. J Cell Sci 118: 889-899, 2005.

Chandrasekaran S, Guo NH, Rodrigues RG, Kaiser J, Roberts DD : Proadhesive and chemotactic activities of thrombospondin-1 for breast carcinoma cells are mediated by integrin and regulated by insulin-like growth factor-1 and CD98. J Biol Chem 274: 11408-11416, 1999.

Deves R, Boyd CA : Surface antigen CD98 (4F2): not a single membrane protein, but a family of proteins with multiple functions. J Membr Biol 173: 165-177, 2000.

Dixon WT, Sikora LK, Demetrick DJ, Jerry LM : Isolation and characterization of a heterodimeric surface antigen on human melanoma cells and evidence that it is the 4F2 cell activation/proliferation molecule. Int J Cancer 45: 59-68, 1990.

Fenczik CA, Sethi T, Ramos JW, Hughes PE, Ginsberg MH : Complementation of dominant suppression implicates CD98 in integrin activation. Nature 390: 81-85, 1997.

Fenczik CA, Zent R, Dellos M, Calderwood DA, Satriano J, Kelly C, Ginsberg MH : Distinct domains of CD98hc regulate integrins and amino acid transport. J Biol Chem 276: 8746-8752, 2001.

Friedl P, Hegerfeldt Y, Tusch M : Collective cell migration in morphogenesis and cancer. Int J Dev Biol 48: 441-449, 2004.

Hato T, Pampori N, Shattil SJ : Complementary roles for receptor clustering and conformational change in the adhesive and signaling functions of integrin $\alpha_{IIb}\beta_3$. J Cell Biol 141: 1685-1695, 1998.

Jeon, JS : Up-regulation of β_1 integrin activity by CD98 signaling. Graduate School Kangwon National University 2004. (in Korean)

Koleniskova TV, Mannion BA, Berditchevski F, Hemler ME : β_1 integrins show specific association with CD98 protein in low density membranes. BMC Biochem 2: 10-18, 2001.

Mitra SK, Hanson DA, Schlaepfer DD : Focal adhesion kinase: in command and control of cell motility. Nat Rev Mol Cell Biol. 6: 56-68, 2005.

Parsons JT, Martin KH, Slack JK, Taylor JM, Weed SA : Focal adhesion kinase: a regulator of focal adhesion dynamics and cell movement. Oncogene 19: 5606-5613, 2000.

Rintoul RC, Buttery RC, Mackinnon AC, Wong WS, Mosher D, Haslett C, Sethi T : Cross-linking CD98 promotes integrin-like signaling and anchorage-independent growth. Mol Biol Cell 13: 2841-2852, 2002.

Zent R, Fenczik CA, Calderwood DA, Liu S, Dellos M, Ginsberg MH : Class-and splice variant-specific association of CD98 with integrin β cytoplasmic domains. J. Biol. Chem. 275: 5059-5064, 2000.

Korean J Phys Anthropol
20(3): 225 ~ 233, 2007

CD98 활성화에 의한 사람유방암세포 MCF-7의 침윤성 증가

**김선미, 김재명, 전준성[1], 안준석, 김대영, 서재성
박경한, 박정현, 김대중, 한장희**

강원대학교 의과대학 해부학교실
[1]인제대학교 의과대학 비뇨기학교실

간추림 : CD98 단백질은 125-kDa 크기의 제2형 막횡단 당단백질로서, β_1 인테그린의 기능을 조절한다. 이는 이 분자가 암세포의 침윤과정에 관여함을 시사한다. 본 연구의 목적은 암세포의 부착과 침윤에 대한 CD98신호의 영향을 조사하는 데에 있다.

MCF-7 세포 표면에서의 CD98의 발현 여부는 면역조직화학법으로 확인하였다. CD98 활성화가 MCF-7 세포의 부착과 침윤에 미치는 영향은 세포-세포밖기질 부착도 및 침윤도측정법을 통하여 조사하였다. Dominant-negative 형태의 FAK 유전자의 일시적 발현을 유도하기 위하여 리포솜 제제를 이용하여 pcDNA3-FAK related non-kinase (FRNK)와 pcDNA3-FAK Y397F plasmid DNA를 각각 세포안으로 도입하였다.

CD98 분자가 단클론항체에 의하여 활성화될 경우, 사람유방암세포주인 MCF-7 세포의 파이브로넥틴, 라미닌과 제4형 콜라겐에 대한 부착력과 침윤력이 증가되었다. EDTA나 β_1 인테그린 억제 항체를 CD98 항체와 함께 처리할 경우, 침윤력에 대한 CD98 효과가 억제되었다. FAK 인산화 억제제인 PP2를 처리하면 CD98에 의해 증가된 MCF-7 세포의 침윤이 감소하였다. 이 결과는 dominant-negative 형태의 FAK 유전자의 과발현을 통하여서도 확인할 수 있었다. 또한, 세포골격단백질의 재구성을 억제하는 약제인 cytochalasin D나 phalloidin도 CD98 신호를 저해하였다. PP2, cytochalasin D나 phalloidin의 CD98 신호 억제효과는 β_1 인테그린의 형태변화를 유도하는 Mn^{++}을 처리할 경우 감소하였다.

본 연구는 CD98분자가 활성화되면 MCF-7 사람유방암세포의 침윤도가 증가되며, 이 과정에 β_1 인테그린의 세포밖기질에의 부착도의 증가, FAK의 인산화와 그에 따른 세포골격의 재구성에 관여함을 보여준다.

찾아보기 낱말 : CD98, β_1 인테그린, 암세포침윤, 세포-세포밖기질 부착, FAK, 액틴, 세포골격

교신저자 : 한장희(강원대학교 의과대학 해부학교실)
전자우편 : parkbs@pusan.ac.kr