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CD109 attenuates TGF-β1 signaling and enhances EGF signaling in SK-MG-1 human glioblastoma cells

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ABSTRACT

CD109 is a glycosylphosphatidylinositol-anchored cell surface protein that is frequently detected in squamous cell carcinomas. CD109 is a negative regulator of TGF-β1 signaling in human keratinocytes, and the N-terminal fragment of CD109 secreted from cells after cleavage by the furin protease is important for modulating TGF-β1 signaling. Previously, we found that CD109 is expressed in human glioblastoma cells; however, the role of CD109 in glioblastoma cells is not established. Here, we describe the effects of CD109 in human glioblastoma cell lines. Three glioblastoma cell lines, SK-MG-1, U251MG and MG178, were tested and CD109 overexpression attenuated TGF-β1 signaling and enhanced EGF signaling in SK-MG-1, but not in U251MG or MG178. The N-terminal CD109 fragment in SK-MG-1 was hyperglycosylated compared with that in MG178 or U251MG. The conditioned medium of CD109-overexpressing SK-MG-1, containing the secreted N-terminal CD109, had a negative effect on TGF-β1 signaling in wild-type SK-MG-1 and MG178, whereas it did not show any effect on EGF signaling. In addition, cell surface CD109 interacts with EGF receptor in SK-MG-1 overexpressing CD109, and exhibited enhanced cell migration and invasion. These findings suggest that CD109 attenuates TGF-β1 signaling and enhances EGF signaling in SK-MG-1 cells and that the membrane-anchored CD109 may play major roles in the EGF signaling pathway.

1. Introduction

CD109 is a glycosylphosphatidylinositol (GPI)-linked glycoprotein located on the plasma membrane [1,2]. It has been studied as a cell surface antigen expressed on some types of normal hematopoietic cells and hematopoietic tumor cells [1,3,4]. CD109 mRNA is highly expressed in several cell lines derived from human tumors, including lung squamous cell carcinomas (SCCs), melanomas and glioblastomas [5]. In tumor tissues, CD109 was immunohistochemically detected in SCCs, urothelial carcinomas, malignant melanomas, basal-like breast carcinomas and epithelial sarcomas [6–12]. CD109 overexpression promotes accelerated cell growth in oral SCC cells. In addition, oral dysplastic epithelia expressing high levels of CD109 progressed to SCC within 3 years at a high frequency, and a strong correlation was detected between CD109 expression and prognosis in soft tissue sarcomas [10,12]. These findings suggest that CD109 might be involved in the development of several human tumors.

CD109 is involved in the TGF-β1 signaling pathway as a negative regulator via formation of a receptor complex with TGF-β receptor I and II in human keratinocytes [13]. CD109 promotes TGF-β receptor internalization and degradation by regulation of SMAD7 and SMURF2 activity in ubiquitin-mediated proteolysis [14,15]. It was also reported that proteolytic processing of CD109 by furin or mesotrypsin is required for its function in cultured cells [16,17]. After cleavage by furin, a proportion of N-terminal 180-kDa

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fragment of CD109 is secreted from cells with the rest remaining on the cell surface. The C-terminal 25-kDa fragment is anchored to GPI on the cell surface. The secreted fragment of CD109 is important for the regulation of TGF-β1 signaling [16]. CD109 is also associated with several nontumorigenic disorders in human and mouse. Aberrant CD109 release from dermal keratinocytes might be a cause of TGF-β1 receptor downregulation and TGF-β1 signal attenuation in psoriasis [18]. CD109 overexpression in the mouse epidermis reduced the inflammatory response and improved collagen organization during wound healing, showing decreased SMAD2/3 phosphorylation [19]. CD109-deficient mice showed that CD109 is necessary for normal growth of the epidermis and appendages [20]. However, the biological significance of CD109 in tumor cells is still unclear.

In this study, we demonstrated that CD109 attenuates TGF-β1 signaling and facilitates EGF signaling in SK-MG-1 human glioblastoma cells, but not U251MG or MG178 cells. The present findings demonstrate the diversity of CD109 function in human tumor cells.

2. Materials and methods

2.1. Plasmid constructions

Construction of the expression vector pcDNA3.1(+)/FLAG-CD109 possessing a FLAG-tagged full-length human CD109 (FLAG-CD109) cDNA was described previously [6]. The retrovirus expression vector pRetroQ/FLAG-CD109 for FLAG-CD109 expression was constructed using pRetroQ (Takara).

2.2. Cell culture and reagents

Human glioblastoma cell lines, SK-MG-1, MG178 and U251MG were maintained in DMEM supplemented with 8% FBS. To generate FLAG-CD109 and control transfectants, SK-MG-1 and U251MG cells were transfected with pcDNA3.1(+)/FLAG-CD109 or pcDNA3.1(+)/FLAG using Lipofectamine 2000 transfection reagent (Invitrogen). Forty-eight hours after transfection, cells were incubated in selection media containing 400 μg/ml G418 (Invitrogen) for 2 weeks, and several stable transfectants were obtained. To produce FLAG-CD109 retroviral supernatants, GP2-293 packaging cells (Takara) were co-transfected with pVSV-G (Takara) and either pRetroQ/FLAG-CD109 or control vector possessing GFP cDNA using FuGENE 6 transfection reagent (Roche). The medium was replaced after 24 h, and virus-containing supernatants were harvested 48 h post-transfection and used to infect MG178 cells. The infected cells were selected in puromycin-containing media (1 μg/ml) for 1 week.

2.3. Signaling analysis

To analyze TGF-β1 or EGF signaling, cells were serum-starved for 2 h and stimulated with 50 pM TGF-β1 (PeproTech) or 5 ng/ml EGF (Invitrogen) for the indicated periods. To analyze the effect of secreted CD109 on TGF-β1 or EGF signaling, SK-MG-1-CD109, SK-MG-1-control, MG178-CD109, and MG178-control cells were grown until confluent and then incubated for another day. The conditioned media of the transfectants were clarified by centrifugation and used for signaling analyses. Wild-type SK-MG-1 and MG178 cells were serum-starved and stimulated with TGF-β1 or EGF in the clarified conditioned media.

2.4. Antibodies

Mouse monoclonal anti-CD109 (C-9) antibody was purchased from Santa Cruz Biotechnology. Mouse monoclonal anti-CD109 (11H3) antibody was purchased from Immuno-Biological Laboratories. Mouse monoclonal anti-FLAG M2 and -β-actin antibodies were purchased from Sigma-Aldrich. Rabbit monoclonal anti-EGFR and -phospho-EGFR (pEGFR) antibodies were from Epitomics. Rabbit polyclonal anti-pAKT and -pERK antibodies and rabbit monoclonal anti-SMAD2 and -pSMAD2 antibodies were from Cell Signaling Technology. Goat anti-rabbit IgG and anti-mouse IgG secondary antibodies conjugated with Alexa 488 or Alexa 594, respectively, were from Invitrogen. The epitope positions on FLAG-CD109 for anti-CD109 and -FLAG antibodies are illustrated in Fig. 1A.

2.5. Glycosylation analyses

For glycosylation inhibition, FLAG-CD109-overexpressing cells were treated with 2 mg/ml tunicamycin for 48 h and used for western blotting (Supplementary Doc S1). For deglycosylation analyses, FLAG-CD109-overexpressing cells were subjected to immunoprecipitation using anti-FLAG antibody (Supplementary Doc S1). Precipitated FLAG-CD109 was treated with PNGase F (Enzymatic Protein deglycosylation kit, Sigma-Aldrich) for 3 h at 37 °C, boiled in 1 × SDS sample buffer, and subjected to western blotting.

2.6. Wound-healing assay

Cells were seeded on 35 mm dishes, and confluent cells were scratched with a disposable plastic pipette tip and allowed to migrate towards the wound. Images were captured with a CCD digital camera (Olympus) coupled to an inverted microscope (Olympus).

2.7. In vitro cell migration and invasion assays

2.5×10^4 cells stained with CellTracker Green CMEDA (Invitrogen) were seeded in the top chamber of HTS FluoroBlok Inserts for migration assay or HTS FluoroBlok Inserts coated with matrigel matrix for invasion assay (8 μm pore size, BD Biosciences) in 200 μl DMEM containing 0.1% FBS. Five hundred microliters of DMEM with 0.1% FBS was added to the bottom chamber. Cells were allowed to migrate for 6 h or invade for 22 h, and cells that had migrated or invaded under the membrane were fixed in 10% formaldehyde. Fluorescence images were captured with a CCD camera (Olympus), and the cells were counted under a fluorescence microscope in five randomly selected fields.

3. Results

3.1. CD109 attenuates TGF-β1 signaling and facilitates EGF signaling in SK-MG-1 cells but not in MG178 and U251MG cells

Transfectants overexpressing FLAG-CD109 were generated using the three glioblastoma cell lines, SK-MG-1, MG178 and U251MG, and TGF-β1 signaling in these and control cells was assessed by western blotting. As expected, SMAD2 phosphorylation after TGF-β1 stimulation was attenuated in CD109-overexpressing SK-MG-1 compared with control cells (Fig. 1C). In contrast, SMAD2 phosphorylation was not affected by CD109 overexpression in MG178 or U251MG. EGF signaling in these cells was then analyzed. After EGF stimulation, phosphorylation of EGFR, AKT, and ERK was enhanced and sustained in CD109-overexpressing SK-MG-1 compared with control cells, whereas it was not affected by CD109 overexpression in MG178 and U251MG (Fig. 1D).

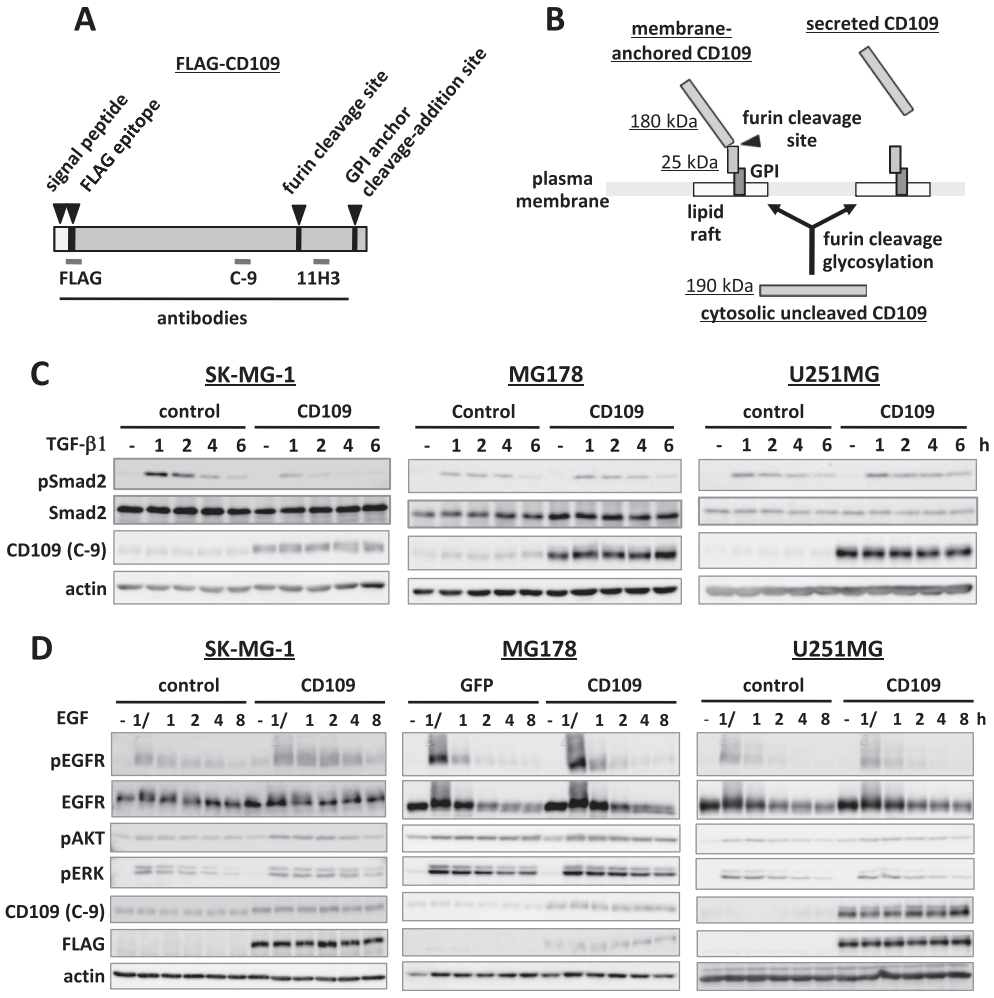

Fig. 1. CD109 attenuates TGF-β1 signaling and enhances EGF signaling in SK-MG-1 cells but not in MG178 and U251MG cells. (A) Protein structure of FLAG-CD109, and epitope positions of antibodies used in this study. FLAG, C-9 and 11H3 indicate anti-FLAG, anti-CD109 (C-9), and anti-CD109 (11H3) antibodies, respectively. (B) A scheme of CD109 processing in cells. Cytosolic uncleaved CD109 (190 kDa) is glycosylated and cleaved by furin, resulting in a C-terminal fragment (25 kDa) anchoring to GPI on the cell surface, and an N-terminal fragment (180 kDa) partially secreted with a fraction binding to the C-terminal fragment. (C, D) SK-MG-1, MG178, and U251MG cells with and without FLAD-CD109 overexpression were stimulated with 50 pM TGF-β1 (C) or 5 ng/ml EGF (D) and downstream signaling was analyzed by western blotting at the times indicated.

3.2. CD109 is hyperglycosylated in SK-MG-1 cells compared with that in MG178 or U251MG cells

To assess why CD109 expression affects TGF-β1 and EGF signaling only in SK-MG-1 cells, CD109 expression in SK-MG-1, U251MG and MG178 cells was analyzed with western blotting. Variable forms of cellular CD109 protein are summarized in Fig. 1B [16]. Two CD109 fragments of 180 and 190 kDa were detected in whole cell lysates using the anti-CD109 (C-9) antibody, whereas two CD109 fragments of 190 and 25 kDa were detected using the anti-CD109 (11H3) antibody (Fig. 2A). The 180-kDa band represents the membrane-anchored N-terminal fragment, the 25-kDa band represents the GPI-anchored C-terminal fragment, and the 190-kDa band represents cytosolic, immature, uncleaved CD109 (Fig. 1B). Interestingly, the endogenous 180-kDa fragment of CD109 in SK-MG-1 cell lysate was a little larger compared with that in U251MG or MG178 (Fig. 2A). FLAG-CD109-overexpressing cell lysates were also subjected to western blotting and immunoprecipitation with anti-FLAG antibody. The expression pattern of exogenous FLAG-CD109 in these cells was similar to that of endogenous CD109 (Fig. 2B, C). Secreted 180 kDa CD109 in the conditioned media was detected by immunoprecipitation and western blotting using the anti-FLAG antibody. The secreted fragment was also a little larger in SK-MG-1 compared with that in U251MG or MG178 (Fig. 2D).

To assess the structural difference between the 180-kDa CD109 fragment in SK-MG-1 and that in U251MG or MG178, the three cell lines were treated with tunicamycin, a glycosylation inhibitor, for 48 h, and their cell lysates were then subjected to western blotting. After the treatment, all of the 190-kDa fragments shifted to approximately 150 kDa, whereas 180- and 25-kDa fragments did not change their size but their amounts were reduced (Fig. 2E). This finding suggests that 150-kDa fragment represents cytosolic uncleaved CD109 without glycosylation, and the 180- and 25-kDa fragments represent the residual undegraded fragments. Next, we treated immunoprecipitated FLAG-CD109 of SK-MG-1-CD109, U251MG-CD109 and MG178-CD109 cells with PNGase F, which cleaves asparagine-linked oligosaccharides from glycoproteins, and the deglycosylated FLAG-CD109 was assessed by western blotting. Two bands, approximately 150 and 130 kDa, were detected by anti-CD109 (C-9) antibody, in which the 150- and 130-kDa fragments might represent deglycosylated, cytosolic, uncleaved CD109 and deglycosylated, membrane-anchored CD109, respectively (Fig. 2F). The sizes of these deglycosylated fragments were similar among the three cell lines. These results indicate that the 180-kDa CD109 in SK-MG-1 is hyperglycosylated compared with that in MG178 or U251MG.

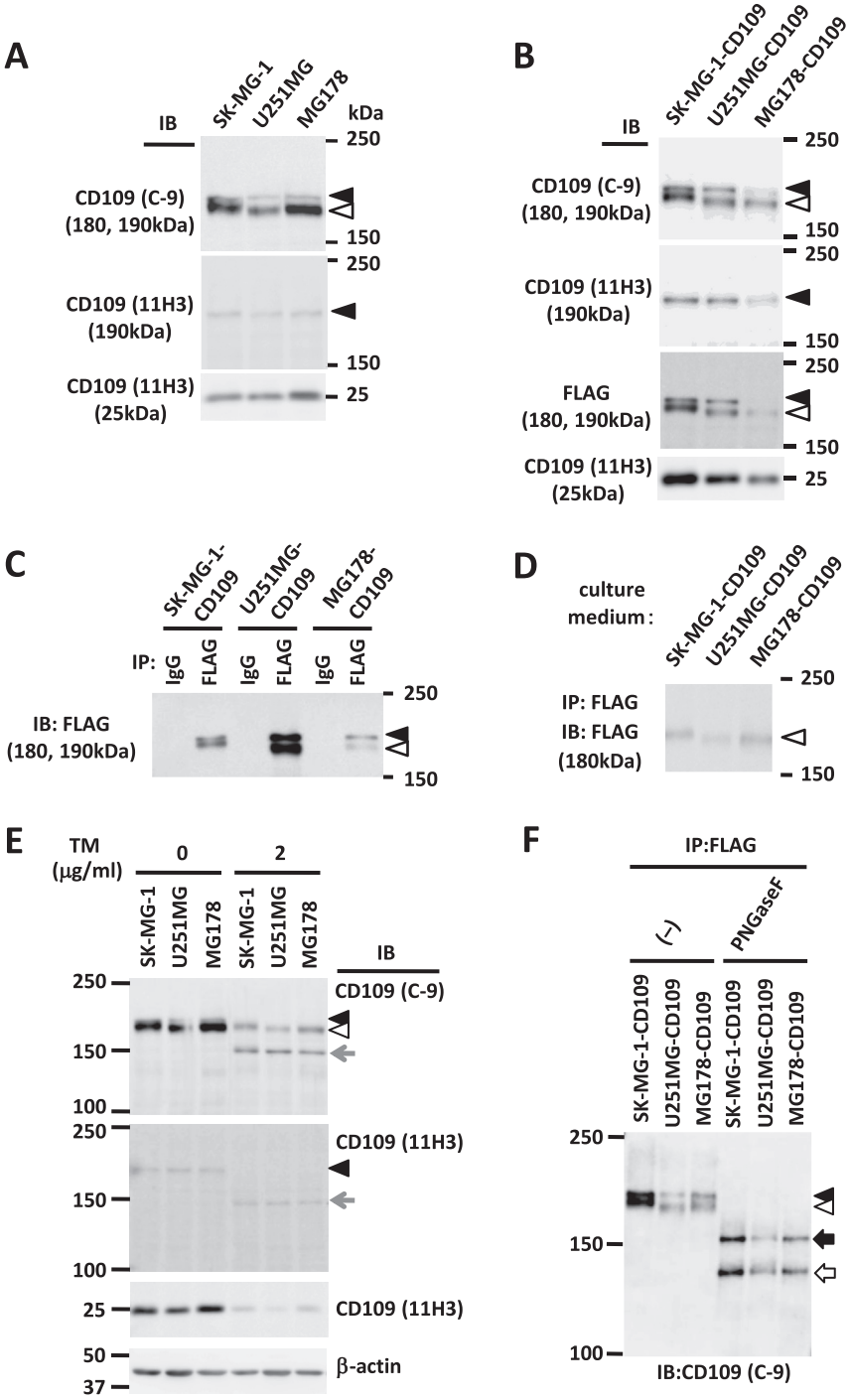

Fig. 2. Endogenous and exogenous CD109 expression in SK-MG-1, MG178 and U251MG cells, and glycosylation analyses of CD109. (A) Western blot analyses for endogenous CD109 expression in SK-MG-1, MG178 and U251MG. (B) Western blot analyses for exogenous CD109 expression in SK-MG-1, MG178, and U251MG overexpressing FLAG-CD109. (C) Immunoprecipitation analysis of CD109. FLAG-CD109 in each cell lysate was immunoprecipitated with anti-FLAG antibody and control IgG, followed by western blotting with anti-FLAG antibody. (D) Secreted CD109 in conditioned media. The conditioned media of SK-MG-1, MG178, and U251MG overexpressing FLAG-CD109 were subjected to immunoprecipitation and western blotting with anti-FLAG antibody. (E) Inhibition of CD109 glycosylation. SK-MG-1, MG178 and U251MG were treated with 2 μg/ml tunicamycin for 48 h, and whole cell lysates from treated and untreated cells were subjected to western blotting. (F) Deglycosylation of CD109. FLAG-CD109 isolated from FLAG-CD109-expressing SK-MG-1, MG178 and U251MG by immunoprecipitation with anti-FLAG antibody was treated with PNGase F for 3 h, and treated and untreated FLAG-CD109 was subjected to western blotting. Closed arrowhead; 190-kDa CD109, open arrowhead; 180-kDa CD109, gray arrow; unglycosylated, uncleaved CD109, closed arrow; deglycosylated, uncleaved CD109, open arrow; deglycosylated, cleaved CD109.

3.3. Secreted CD109 from SK-MG-1 cells modulates TGF-β1 signaling but not EGF signaling

Since it has been reported that the secreted N-terminal fragment of CD109 is important for TGF-β1 signal modulation, we next examined whether the N-terminal CD109 fragment secreted from SK-MG-1 is associated with EGF signal enhancement as well as TGF-β1 signal attenuation. When wild-type SK-MG-1 were stimulated with TGF-β1 in SK-MG-1-CD109 conditioned media, SMAD2 phosphorylation was attenuated compared with stimulation in SK-

MG-1-control conditioned media, as expected (Fig. 3A). The signal attenuation was also observed when wild-type MG178 were stimulated with TGF-β1 in the same way. In contrast, when wild-type SK-MG-1 and MG178 were stimulated with TGF-β1 in MG178-CD109 conditioned media, the signal modulation was not observed (Fig. 3B). These results suggest that the N-terminal CD109 fragment secreted from SK-MG-1-CD109 cells, but not from MG178-CD109 cells, modulates TGF-β1 signaling. We also examined the ability of the secreted CD109 from SK-MG-1-CD109 cells to modulate EGF signaling; however, EGF signal modulation in wild-type SK-MG-1 was not observed after EGF stimulation (Fig. 3C). These results indicate that secreted CD109 may play a role in TGF-β1 signal attenuation but not for EGF signal enhancement, and that the mechanism of EGF signal modulation by CD109 is different from that of TGF-β1 signal modulation.

3.4. CD109 interacts with the EGF receptor in SK-MG-1 cells

In this study, we observed a novel finding that CD109 is involved in EGF signaling in SK-MG-1. To elucidate the molecular mechanism of CD109 involvement in the EGF signaling pathway in SK-MG-1, we assessed the interaction between CD109 and EGFR. When FLAG-CD109 was immunoprecipitated from SK-MG-1-CD109 cell lysate with anti-FLAG antibody, endogenous EGFR was co-precipitated (Fig. 4A). The amount of EGFR co-precipitated with FLAG-CD109 was reduced after EGF stimulation. Co-precipitated EGFR was hardly detected when MG178-CD109 or U251MG-CD109 cell lysates were used for the co-immunoprecipitation analysis (data not shown).

We further assessed the intracellular localization of CD109 and EGFR in SK-MG-1-CD109 cells by immunocytochemistry (Supplementary Doc S1). FLAG-CD109 was localized to the plasma membrane and colocalized with EGFR in the absence of EGF stimulation (Fig. 4B). Ten minutes after EGF stimulation, the majority of EGFR was internalized to the cytoplasm; however, CD109 was still on the plasma membrane and colocalized with a small fraction of EGFR (Fig. 4B). No apparent difference in EGFR internalization was observed between SK-MG-1-CD109 and SK-MG-1-control cells. These results indicate that CD109 interacts with EGFR in the absence of EGF stimulation and that the interaction might be abolished upon phosphorylation of EGFR by EGF stimulation; furthermore CD109 does not affect EGFR internalization.

3.5. CD109 enhances migration and invasion of SK-MG-1 cells

Last, we assessed the effect of CD109 on motility of SK-MG-1. In the *in vitro* wound-healing assay, SK-MG-1-CD109 cells migrated to the wound area much faster than SK-MG-1-control cells (Fig. 4C). The SK-MG-1-CD109 wound was almost closed 22 h after scratching, whereas the SK-MG-1-control wound was still a considerable size. The counts of migrated SK-MG-1-CD109 cells were significantly higher compared with those of SK-MG-1-control cells in the Boyden Chamber assay (Fig. 4D). We then analyzed the invasion ability of SK-MG-1-CD109 and SK-MG-1-control cells. Fig. 4E shows the images of fluorescence-labeled cells that had invaded to the underside of the Boyden Chamber membrane through the matrigel matrix. The counts of invaded SK-MG-1-CD109 cells were higher than those of SK-MG-1-control cells (Fig. 4F), demonstrating that CD109-overexpressing SK-MG-1 invaded much faster than control cells. These results indicate that CD109 facilitates motility in SK-MG-1.

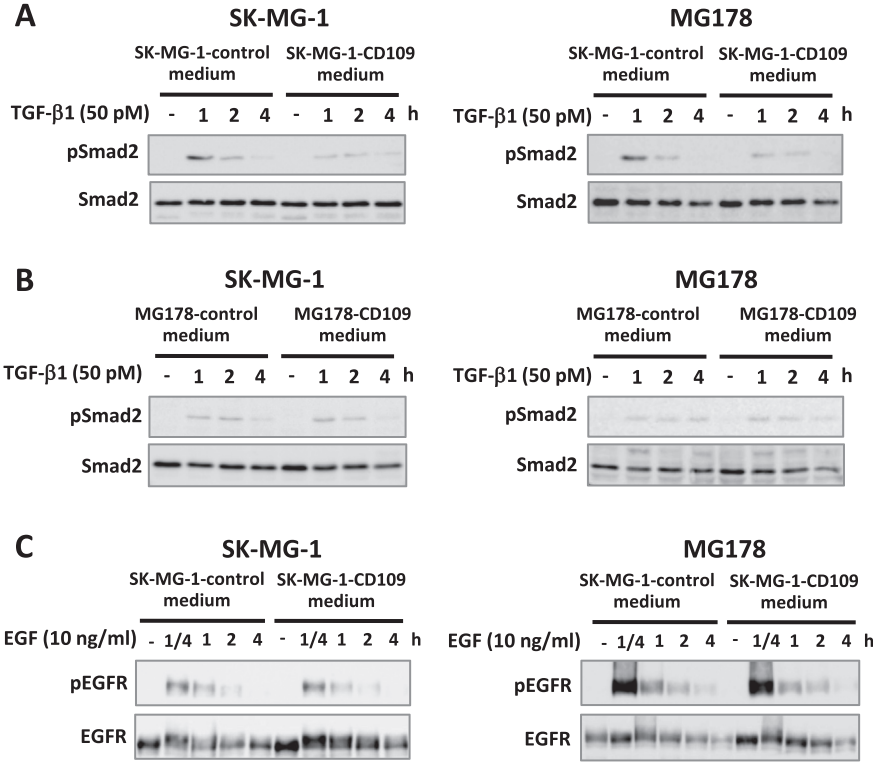

Fig. 3. Secreted CD109 from SK-MG-1 cells modulates TGF-β1 signaling, but not EGF signaling. (A, B) FLAG-CD109 secreted from SK-MG-1-CD109, but not from MG178-CD109, inhibited TGF-β1 signaling. Wild-type SK-MG-1 and MG178 cells were stimulated with 50 pM TGF-β1 in the conditioned media from SK-MG-1-CD109 or SK-MG-1-control cells (A) and MG178-CD109 or MG178-control cells (B) for the indicated times, and phosphorylation of SMAD2 was assessed by western blotting. (C) FLAG-CD109 secreted from SK-MG-1 cells did not enhance EGF signaling. Wild-type SK-MG-1 and MG178 cells were stimulated with 10 ng/ml EGF in the conditioned media from SK-MG-1-CD109 or SK-MG-1-control cells for the indicated times, and phosphorylation of EGFR was assessed by western blotting.

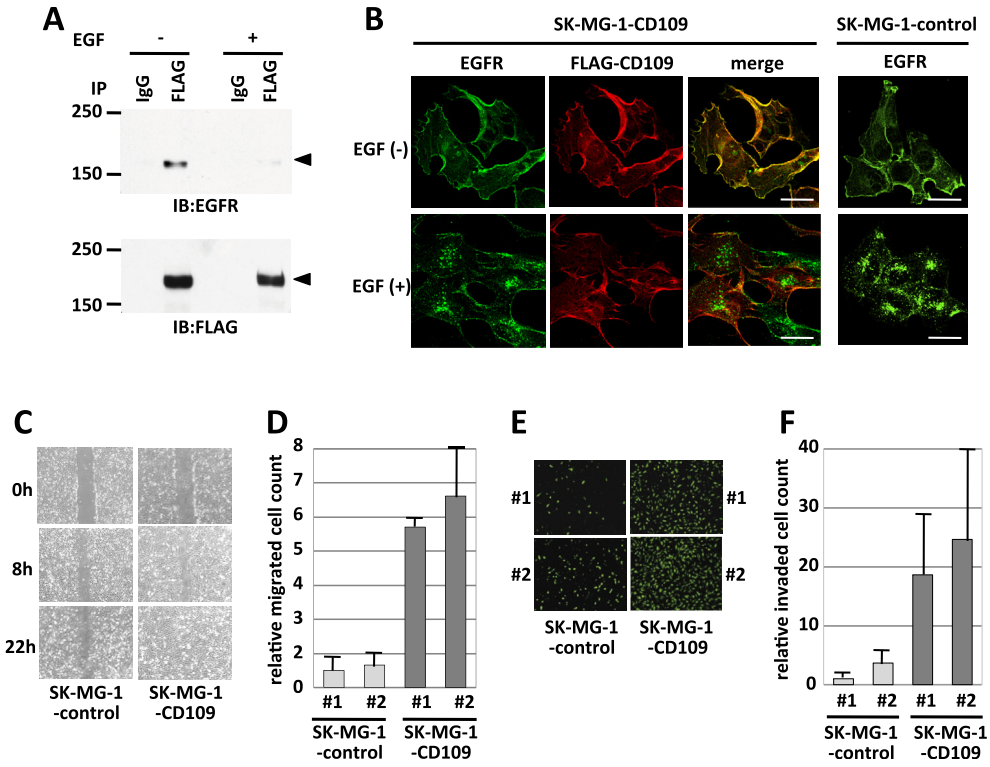

Fig. 4. CD109 interacts with EGF receptor and enhances cell migration and invasion in SK-MG-1 cells. (A) CD109 interacts with EGFR. Cell lysates from SK-MG-1-CD109 cells with or without EGF stimulation (25 ng/ml, for 15 min) were subjected to immunoprecipitation with anti-FLAG antibody and control IgG followed by western blotting. (B) CD109 colocalizes with EGFR in the absence of EGF stimulation. SK-MG-1-CD109 and SK-MG-1-control cells with or without EGF stimulation (25 ng/ml, for 10 min) were fluorescently double-immunostained with rabbit anti-EGFR and mouse anti-FLAG antibodies (Supplementary Doc S1). Scale bars indicate 20 μm in length. (C, D) CD109 enhances cell migration. (C) SK-MG-1-CD109 and SK-MG-1-control cells were subjected to the wound-healing assay, and cell images were captured at 0, 8 and 22 h after commencing the assay. (D) Two stable SK-MG-1-CD109 and SK-MG-1-control clones were subjected to an *in vitro* migration assay. The number of migrated SK-MG-1-control #1 cells was defined as 1.0. $^{*}p < 0.05$. (E, F) CD109 enhances cell invasion ability. (E) Images of invaded SK-MG-1-control and SK-MG-1-CD109 cells. (F) Relative number of invaded cells for each cell line is shown. The number of invaded SK-MG-1-control #1 cells is defined as 1.0. $^{**}p < 0.05$.

4. Discussion

Several genetic and epigenetic alterations are involved in glioblastoma development [21,22]. *EGFR* amplification and overexpression are detected in approximately 40% and more than 60% of primary glioblastomas, respectively. A mutated *EGFR* transcript, *EGFRvIII*, in which exons 2–7 are deleted, is present in 20–50% of glioblastomas with *EGFR* amplification. The EGFRvIII protein lacking the ligand-binding domain is constitutively activated without ligand stimulation, and enhances cell proliferation through the PI3K/AKT pathway. Thus, signaling status through EGFR is a crucial factor in the pathogenesis of glioblastoma.

We and others have previously detected CD109 expression in human glioblastoma cells by northern blot and RT-PCR analyses [5,23]. We aimed to investigate the significance of CD109 expression in glioblastoma cells. We showed that CD109 enhances EGF signaling in SK-MG-1 cells via a mechanism that is different from its involvement in TGF-β1 signaling. In addition, CD109 facilitated cell migration and invasion in SK-MG-1. These results suggest that CD109 is associated with tumorigenesis in a portion of glioblastomas. EGFR alterations are detected in many kinds of cancer, including non-small cell lung cancers, head and neck SCCs, renal cell carcinomas, breast cancers, and gastrointestinal cancers [24–27], and CD109 is highly expressed in several cancers, especially SCCs [6–12]. However, the significance of CD109 in cancer biology is unclear. The present results demonstrate that CD109 is associated with growth factor signaling in glioblastoma, which might be applicable to other cancers expressing CD109.

It is revealed that the effect of CD109 on EGF and TGF-β1 signaling is cell-type dependent in glioblastoma cells. CD109 enhanced EGF signaling and attenuated TGF-β1 signaling in SK-MG-1, but not in MG178 and U251MG. In addition, N-terminal CD109-containing conditioned media from SK-MG-1-CD109 cells induced TGF-β1 signal attenuation in wild-type SK-MG-1 and MG178, whereas that from MG178-CD109 cells did not. The N-terminal fragment of CD109 in SK-MG-1 is hyperglycosylated compared with that in MG178 or U251MG. These results suggest that CD109 activity on EGF and TGF-β1 signaling may be associated with glycosylation of the N-terminal CD109 fragment. CD109 shows various glycosylation levels dependent on tissues or tumor cells [Y. Murakumo, unpublished data, [20,28]], which suggests that CD109 activity may be diverse in various tumor cells. A recent publication reported that variability of vascular endothelial growth factor (VEGF)-targeted therapies is dependent on glycosylation status of the endothelial cells [29]. They showed that anti-VEGF-sensitive tumors exhibit high levels of α2-6-linked sialic acid on tumor vessels, which inhibits galectin-1 binding to endothelial cells. Meanwhile anti-VEGF-refractory tumors show decreased α2-6-linked sialylation in tumor-associated vasculature, which facilitates VEGFR2 activation and confers resistance to anti-VEGF therapy. Therefore, it is possible that the glycosylation diversity of CD109 might be associated with glioblastoma biology and the therapeutic effect of EGFR targeting therapy.

CD109 is cleaved by furin and a fraction of the resultant N-terminal fragment of CD109 is secreted from cells; the rest remains on the cell surface. Expression of the soluble form of CD109 attenuated

TGF-β1 signaling in HEK293 cells, and addition of recombinant soluble CD109 to culture media attenuated TGF-β1 signaling and enhanced phosphorylation of STAT3 [16,18], indicating that the secreted form of CD109 is important for signal transduction in the TGF-β1 and STAT pathways. Compatible with these previous reports, we demonstrated a negative effect of the secreted N-terminal CD109 fragment in TGF-β1 signaling; however, it did not modulate EGF signaling. In addition, we demonstrated that the membrane-anchored N-terminal CD109 fragment interacts with EGFR, suggesting that membrane-anchored N-terminal CD109 may play an important role in EGF signal enhancement. In the TGF-β1 signaling pathway, it is supposed that secreted CD109 binds to TGF-β1 and then binds to TGF-β receptor I and II forming a receptor complex for TGF-β1 signal transduction [13]. In the EGF signaling pathway, we propose that CD109 on the cell surface forms a complex with EGFR and enhances phosphorylation of EGFR upon EGF stimulation, and that the interaction between CD109 and EGFR is abolished after EGF stimulation. Thus, CD109 in SK-MG-1 cells is involved in both TGF-β1 and EGF signaling through different processes, using secreted CD109 and membrane-anchored CD109, respectively; both forms of CD109 play important roles in cell biology.

In conclusion, our results indicate that CD109 is involved in both EGF and TGF-β1 signaling, two major molecular pathways in human glioblastoma development. A precise elucidation of the effect of CD109 on EGF and TGF-β signaling is necessary for understanding glioblastoma biology.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.093.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.093.

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