Transactivation of the Epidermal Growth Factor Receptor by Formylpeptide Receptor Exacerbates the Malignant Behavior of Human Glioblastoma Cells

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Abstract

The G protein-coupled formylpeptide receptor (FPR), which mediates leukocyte migration in response to bacterial and host-derived chemotactic peptides, promotes the chemotaxis, survival, and tumorigenesis of highly malignant human glioblastoma cells. Because glioblastoma cells may also express other receptors for growth signals, such as the epidermal growth factor (EGF) receptor (EGFR), we investigated the role of EGFR in the signaling cascade of FPR and how two receptors cross-talk to exacerbate tumor growth. We found that N-formyl-methionyl-leucyl-phenylalanine, an FPR agonist peptide, rapidly induced EGFR phosphorylation at tyrosine residue (Tyr) 992, but not residues 846, 1068, or 1173, in glioblastoma cells, whereas all these residues were phosphorylated after only EGF treatment. The FPR agonist-induced EGFR phosphorylation in tumor cells was dependent on the presence of FPR as well as Goi proteins, and was controlled by Src tyrosine kinase. The transactivation of EGFR contributes to the biological function of FPR in glioblastoma cells because inhibition of EGFR phosphorylation significantly reduced FPR agonist-induced tumor cell chemotaxis and proliferation. Furthermore, depletion of both FPR and EGFR by short interference RNA abolished the tumorigenesis of the glioblastoma cells. Our study indicates that the glioblastoma-promoting activity of FPR is mediated in part by transactivation of EGFR and the cross-talk between two receptors exacerbates the malignant phenotype of tumor cells. Thus, targeting both receptors may yield antiglioblastoma agents superior to those targeting one of them. [Cancer Res 2007;67(12):5906–13]

Introduction

Formylpeptide receptor (FPR) is a G protein-coupled receptor (GPCR), originally identified in phagocytic leukocytes, which mediates cell chemotaxis and activation in response to the bacterial chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (fMLF). Agonist binding to FPR elicits a cascade of signal transduction pathways that involve phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinases (MAPK), and the transcription factor nuclear factor-κB (for review, see ref. 1).

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Materials and Methods

Reagents. FMLF was purchased from Sigma-Aldrich. Antibodies against phosphorylated EGFR (at tyrosine 922, 845, 1068, and 1173) and extracellular signal-regulated kinases 1/2 (ERK1/2) were from Cell Signaling Technology. The EGFR-specific tyrosine kinase inhibitor AG1478 was from Calbiochem. The Src tyrosine kinase inhibitor PP2 [4-aminophenyl)-(7-(7-butyl)pyrazolo[3,4-d] pyrimidine] was from Calbiochem. Neutralizing antibodies against EGFR and the extracellular domain of EGFR were from Santa Cruz Biotechnology. Human glioblastoma cell line U87 was obtained from the American Type Culture Collection. The cells were grown in DMEM containing 10% FCS and antibiotics.

Chemotaxis. Chemotaxis assays were done in 48-well chemotaxis chambers (NeuroProbe; refs. 10, 15). A 27-μl aliquot of chemoattractants was placed in the wells of the lower compartment, and 50 μl of tumor cells (at 3 × 10^5/mL) were placed in the wells of the upper compartment. The upper and lower compartments of the chemotaxis chambers were separated by a 10-μm-pore polycarbonate filter (GE Osmonics Labstore) coated with collagen type I (BD Biosciences) at 50 μg/mL. After incubation at 37°C for 270 min, the filters were removed and stained, and cells that migrated across the filters were counted by light microscopy. The results were expressed as the mean number (±SE) of migrated cells in three high-powered fields (×400 magnification) in triplicate samples or as chemotaxis index representing the fold increase in cell migration in response to stimulants over medium control.

Immunoblot. Tumor cells were lysed in 150 μl of 1× SDS sample buffer [62.5 mmol/L Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, and 50 mmol/L DTT], sonicated for 3 s, and then boiled for 5 min. The cell lysate was then centrifuged at 10,000 × g at 4°C for 10 min. Total protein was electrophoresed on 4% to 12% gradient Tris-glycine precast gels (Invitrogen) and transferred onto Immunoblot P membranes (Millipore). The membranes were blocked by incubation in 5% nonfat dry milk for 1 h at room temperature and then incubated with primary antibodies in PBS containing 0.01% Tween 20 overnight at 4°C. After incubation with a horseradish peroxidase–conjugated secondary antibody, the protein bands were detected with Super Signal Chemiluminescent Substrate Stable Peroxide Solution (Pierce) and BIOMAX-MR film (Eastman Kodak). When necessary, the membranes were stripped with Restore Western Blot Stripping Buffer (Pierce) and reprobed with antibodies against various cellular proteins.

Cell proliferation. Tumor cells were cultured in 12-well tissue culture plates at 2 × 10^5 per well in DMEM containing 10% FCS for 12 h. After a 1-h treatment with various inhibitors, the cells were incubated in DMEM containing 0.5% FCS alone or in the presence of 1 μmol/L FMLF or 10 ng/mL EGF. After incubation at 37°C for different times, the cells were detached by 0.5% trypsin–EDTA and counted. The results were expressed as mean number (±SE) of cells in six replicates. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were also done by Cell Growth Determination kit (Sigma) to assay the proliferation of U87 glioblastoma cells. Briefly, cells cultured in 96-well plates were incubated with MTT solution at 100 μl for 3 h. The supernatants were then removed, and 100 μl MTT solvent were added. After gentle pipetting, absorbance was measured in a spectrometer at 570 nm wavelength. After subtraction of the absorbance at 690 nm, the results were expressed as the mean proliferation units (±SE) obtained from six replicates.

Ca^2+ flux. Ca^2+ mobilization was measured by incubating 2 × 10^5 tumor cells in 1 mL in loading medium (DMEM containing 10% FCS and 2 mmol/L glutamine) with 7 μmol/L Fura-2 acetoxymethyl ester (Molecular Probes) for 45 min at room temperature. The dye-loaded cells were washed and resuspended in saline buffer [138 mmol/L NaCl, 6 mmol/L KCl, 1 mmol/L CaCl_2, 10 mmol/L HEPES, 5 mmol/L glucose, and 0.1% bovine serum albumin (pH 7.4)] at a density of 0.5 × 10^6/mL. The cells were then transferred into quartz cuvettes (1 × 10^6 cells in 2 mL of saline buffer), and the cuvettes were placed in a fluorescence spectrometer (Perkin-Elmer). Stimulants were added to the cuvettes in a volume of 20 μL, and the intensity of the fluorescence was measured by the use of the ratio of the absorbance at 340 to 380 nm, calculated with an FL WinLab  program (Perkin-Elmer).

Detection of EGF dimer. After stimulation with EGF or FMLF, U87 cells were washed twice with ice-cold PBS containing 0.33 mmol/L MgCl_2 and 0.9 mmol/L CaCl_2 ([PBS+] and) and chemically cross-linked for 30 min at room temperature with freshly prepared 5 mmol/L bis(sulfosuccinimidyl) suberate (Pierce). The reaction was terminated by further incubating the cells with 200 mmol/L glycine for 5 min. The cells were washed twice with ice-cold PBS (+) and lysed with 1× SDS. The lysate was centrifuged at 20,000 × g for 10 min, then equal amount of proteins were separated by 6% Tricine–SDS-PAGE and subjected to immunoblot to detect EGF.

Generation of U87 cells stably expressing EGF siRNA. The generation of U87 cells transfected by FPRsiRNA was described previously (10, 16). The FPRsiRNA-transfected cells were further transfected by retrovirus containing blank or EGF siRNA expression cassettes. A hairpin 19-nucleotide sequence was targeted to EGFR mRNA at the nucleotides 645 to 663 (5'-GGACTGGCAGTCTGAAAT-3'). Genbank accession no. NM_005228. Retroviral vector stocks were produced by transient transfection of Phoenix-Ampho cells with the Superfect Transfection Reagent (Qiagen) and 5 μg of siRNA expression plasmid (pRNAi-H1.4/Retro, SD1254, Genscript). The virus was collected from the culture supernatants on day 2 after transfection, and the cells were transfected with the retroviral vectors in the presence of polybrene at 5 μg/mL. The stably transfected U87 cells containing expression cassettes of EGFRsiRNA (EsriRNA), FPRsiRNA (FsiRNA), or both siRNA (EsFsiRNA) were selected and maintained by incubation with 2 μg/mL puromycin and 200 ng/mL hygromycin (BD Biosciences).

Tumor microsphere formation. Anchorage-independent growth of U87 cells containing EsriRNA, FsiRNA, EsiRNA, or blank cassettes (Mock) were examined in a semisolid agar. DMEM containing 10% FCS was warmed to 48°C and diluted with Bacto-Agar to make a 0.6% (w/v) solution. The solution (300 μL) was poured into 24-well culture plates and allowed to solidify for 15 min. A volume of 200 μL of 0.3% agar solution containing 2,500 tumor cells were then layered over the bottom agar in each well. The plates were incubated in 5% CO_2 at 37°C, with a medium change every other day. After 3 weeks, tumor colonies were recorded under light microscopy.

Tumorigenesis. U87 cells containing EsriRNA, FsiRNA, or EsiRNA were implanted s.c. into the flank of 4-week-old (20–22 g) female athymic Ncrnu/nu mice [National Cancer Institute (NCI) Animal Production Program, NCI-Frederick, Frederick, MD] at 5 × 10^7 per mouse in 100 μL of PBS. The growth of implanted tumors was examined every other day. Tumor size was calculated by the formula lw^2/2, where l is the length of the tumor and w is the width of each group contained at least five mice in one experiment. Nontransfected U87 cells and cells transfected with random siRNA (mock) were used as controls. The results were expressed as the mean (±SE) number of colonies counted in triplicates. NCI-Frederick is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and follows the Public Health Service Policy for the Care and Use of Laboratory Animals. Animal care was provided in accordance with the procedures outlined in the Guide for Care and Use of Laboratory Animals (1996, National Research Council, National Academy Press, Washington, DC).

Statistical analyses. All experiments were done at least thrice. A computer-aided t test program SPSS (version 11.0; GraphPad Software, Inc.) was used to determine the statistical significance of the difference between cell responses to testing materials versus controls. The statistical significance of EGF-induced tumor cell chemotaxis was analyzed with ANOVA. P ≤ 0.05 were considered statistically significant.

Results

Glioblastoma cells express functional EGFR. We first tested for the presence of functional EGFR on human glioblastoma cell line U87, which expresses the GPCR FPR. Figure 1 shows that U87 cells migrated in response to EGF at low ng/mL concentration range (Fig. 1A). At higher ng/mL concentrations, EGF also induced a robust Ca^2+ flux in U87 cells. In addition, EGF induced EGF
phosphorylation at tyrosine residue Tyr$^{992}$ (Fig. 1B and C). In parallel experiments, we confirmed that U87 cells responded to the FPR agonist peptide fMLF by chemotaxis and Ca$^{2+}$ flux (Fig. 1D). Thus, U87 concomitantly expresses functional FPR and EGFR.

**FPR agonist fMLF induces EGFR phosphorylation.** We next examined the capacity of FPR to transactivate EGFR in U87 cells. Figure 2 shows that stimulation of the tumor cells with EGF resulted in the phosphorylation of EGFR at Tyr$^{845}$, Tyr$^{1173}$, Tyr$^{1068}$, in addition to Tyr$^{992}$ in a time- and concentration-dependent manner with maximal phosphorylation of all tyrosine residues occurring at 2 min with 10 ng/mL EGF (Fig. 2A). fMLF also induced EGFR phosphorylation; however, in contrast to the effect of EGF, only Tyr$^{992}$ in EGFR underwent a rapid and persistent phosphorylation in response to fMLF at an optional concentration of 100 nmol/L (Fig. 2B–C).

**fMLF-induced EGFR Tyr$^{992}$ phosphorylation is mediated by FPR.** The fMLF-induced EGFR Tyr$^{992}$ phosphorylation was mediated by the receptor FPR, because pertussis toxin, an inhibitor of the Gs proteins coupled to FPR, but not cholera toxin, a Gs protein inhibitor, abolished the effect of fMLF (Fig. 3A).
In contrast, both pertussis toxin and cholera toxin failed to inhibit EGF-induced EGFR phosphorylation (Fig. 3A). The need for FPR in fMLF-induced EGFR Tyr992 phosphorylation was further confirmed by using U87 cells transfected with FPRsiRNA (10), which lost the capacity to migrate in response to fMLF and also failed to undergo EGFR Tyr992 phosphorylation (Fig. 3B and C). In contrast, the same FPRsiRNA containing U87 cells exhibited normal chemotaxis and EGFR Tyr992 phosphorylation in response to EGF (Fig. 3B and C). These results show the requirement for FPR to mediate fMLF-induced EGFR Tyr992 phosphorylation in U87 glioblastoma cells.

In an effort to elucidate the mechanistic basis for EGFR Tyr992 phosphorylation induced by FPR agonist peptide fMLF, we investigated whether fMLF-stimulated glioblastoma cells produced EGF, which, in turn, activated EGFR in an autocrine loop. Figure 4 shows that a neutralizing anti-EGFR antibody was

![Figure 3](Image 1)

**Figure 3.** Involvement of FPR and Gαi proteins in fMLF-induced EGFR Tyr992 phosphorylation. A, U87 cells were preincubated with 500 ng/mL pertussis toxin (PTX, an inhibitor of Gαi protein) or 500 ng/mL cholera toxin (CTX, an inhibitor of Gαs protein) at 37°C for 60 min, then were stimulated with fMLF (100 nmol/L, 10 min) or EGF (10 ng/mL, 2 min). The cell lysates were measured for EGFR Tyr992 phosphorylation. Densitometric measurement of phosphorylated Tyr992 was done using Image J program after normalization against total EGFR protein (EGFR). B, chemotaxis of mock or FPR siRNA (FsiRNA)–transfected U87 cells in response to fMLF or EGF. *, P < 0.01, significantly reduced cell migration compared with mock-transfected cells (Mock). BM, medium. C, phosphorylation of EGFR Tyr992 induced by EGF (10 ng/mL, 1 min) or fMLF (100 nmol/L, 10 min) in mock or FsiRNA-transfected U87 cells.

![Figure 4](Image 2)

**Figure 4.** The role of EGF and Src kinase in fMLF-induced EGFR phosphorylation and dimerization of EGFR. A, blockage of EGF-induced, but not fMLF-induced, U87 cell chemotaxis by anti-EGFR antibody. U87 cells were preincubated with anti-EGFR (1 μg/mL) at 37°C for 30 min, then were measured for chemotaxis in response to fMLF or EGF. *, P < 0.01, significantly reduced cell migration compared with IgG-treated U87 cells. B, inhibition of EGF-induced, but not fMLF-induced phosphorylation by anti-EGFR and anti-EGF antibodies. U87 cells were preincubated with 1 μg/mL anti-EGFR antibody or anti-EGF antibody at 37°C for 30 min; the cells were then stimulated with fMLF or EGF (10 ng/mL, 2 min) and measured for EGFR Tyr992 phosphorylation. In parallel experiments, EGF (10 ng/mL) or fMLF (100 nmol/L) was preincubated with an anti-EGF antibody (1 μg/mL) for 30 min at 37°C. The mixtures were then added to cell culture [EGF/anti-EGF, 2 min; fMLF/anti-EGF, 10 min] before measurement of EGFR Tyr992 phosphorylation. C, inhibition of fMLF-induced EGFR Tyr992 phosphorylation by the Src kinase inhibitor PP2. U87 cells were preincubated with 20 μmol/L PP2 at 37°C for 30 min, then were stimulated with EGF (10 ng/mL, 2 min) or fMLF (100 nmol/L, 10 min) and measured for Tyr992 phosphorylation. D, U87 cells were treated with 10 ng/mL EGF for 2 min or 100 nmol/L fMLF for 10 min. After two washes with ice-cold PBS (+), the cells were treated with the protein cross-linker bis(sulfosuccinimidyl) suberate (BS3). The cell lysates were separated by 6% Tris-glycine SDS-PAGE and subjected to immunoblot with anti-EGFR antibody. Densitometry denotes the ratio of EGFR dimmers/monomers.
capable of abolishing EGF-induced U87 cell chemotaxis and the phosphorylation of EGFR Tyr 992 (Fig. 4A and B). However, this anti-EGFR antibody failed to show any effect on fMLF-induced U87 cell migration or phosphorylation of EGFR Tyr 992 (Fig. 4A and B). In addition, whereas the anti-EGFR antibody completely inhibited EGF-induced EGFR Tyr 992 phosphorylation in U87 cells, it did not show any effect on fMLF-induced phosphorylation of EGFR Tyr 992 (Fig. 4B). These results suggest that phosphorylation of EGFR Tyr 992 in response to fMLF is not dependent on the release of EGF by FPR agonist-stimulated tumor cells.

We subsequently examined the signal transduction pathways coupled to FPR that might be responsible for the phosphorylation of EGFR Tyr 992. In addition to the Gαi proteins, which were essential for the effect of FPR as shown by inhibition with the Gαi-specific inhibitor pertussis toxin (Fig. 3A), we found that an inhibitor of the Src tyrosine kinase PP2 completely inhibited fMLF-induced, but not EGF-induced, EGFR Tyr 992 phosphorylation (Fig. 4C). In contrast, the inhibitors of protein kinase C or PI3K did not block the effect of fMLF on EGFR Tyr 992 phosphorylation (data not shown). We therefore conclude that Src kinase plays a key role in bridging the signal transduction from FPR to EGFR Tyr 992. We additionally found that activation of FPR in U87 cells induced rapid dimerization of EGFR (Fig. 4D), an evidence further supporting the transactivation of EGFR by FPR agonist peptide fMLF.

FPR and EGFR cooperate to promote the motility and growth of glioblastoma cells. To investigate the biological significance of EGFR Tyr 992 phosphorylation in FPR-mediated glioblastoma cell chemotaxis and growth, we used AG1478, a specific tyrosine kinase inhibitor of activated EGFR. We found that both fMLF- and EGF-induced EGFR Tyr 992 phosphorylation was inhibited when U87 cells were treated with AG1478 (Fig. 5A).

Although AG1478-treated U87 cells failed to respond to EGF by phosphorylation of ERK1/2 MAPK, chemotaxis, and proliferation, AG1478 partially but also significantly inhibited fMLF-induced ERK1/2 phosphorylation, chemotaxis, and proliferation of the tumor cells (Fig. 5A–C). Interestingly, although AG1478-treated U87 cells lost the capacity to respond to EGF by Ca2+ flux (Fig. 5D), they showed normal Ca2+ mobilization to fMLF (Fig. 5D). Thus, EGFR Tyr 992 transactivation participates in FPR-mediated signaling pathways that promote glioblastoma cell chemotaxis and proliferation. In contrast, the FPR signaling pathways mediating Ca2+ mobilization is independent of EGFR transactivation.

We then explored the effect of cross-talk between FPR and EGFR on the malignant behavior of glioblastoma cells. In a soft-agar tumor microsphere formation model, we found that both wild-type and mock-transfected U87 cells rapidly formed numerous tumor spheres (Fig. 6A). U87 cells containing either FPRsiRNA or EsiRNA each showed significantly (P < 0.01) retarded formation of tumor spheres (Fig. 6B) as measured by the sphere numbers and size. Knocking down both FPRsiRNA further diminished the capacity of U87 cells to form larger (50–200 µm) spheres. These results were consistent with in vitro cell growth curves showing the proliferation of U87 cells with either FsiRNA or EsiRNA to be diminished, whereas combination of both siRNAs further reduced the rate of cell growth (Fig. 6C).

We then injected human glioblastoma cells s.c. into the flanks of athymic mice and measured the rate of tumor formation and growth. Tumor nodules appeared in all mice injected with wild-type U87 or mock-transfected U87 cells by day 5. In contrast, 60% of the mice implanted U87 cells containing EsiRNA or FsiRNA grew visible tumors only by day 21 after implantation. None of the mice implanted with U87 cells containing both EsiRNA and FsiRNAs.
developed tumors by day 33 posttransplantation (Fig. 6D). In addition, tumors formed by wild-type or mock-transfected cells grew more rapidly than those formed by cells containing either EsiRNA or FsiRNA (Fig. 6D; Supplementary Fig. S1). These results indicate that both FPR and EGFR play important roles in the tumor cell growth and tumorigenesis, and two receptors cooperate to potentiate the malignant behavior of the glioblastoma cells.

**Discussion**

**FPR in U87 glioblastoma cells transactivates EGFR.** In this study, we showed that FPR in human glioblastoma cells transduces signals that selectively induce the phosphorylation of EGFR Tyr992, which is partially responsible for the chemotactic and growth-stimulating effect of FPR agonist peptide fMLF on tumor cells. To our knowledge, this is the first demonstration that FPR, a GPCR that is implicated in inflammation and host defense against microbial infection mediated by cells of the innate immunity (1), is capable of engaging EGFR in promoting glioblastoma cell growth and tumorigenesis.

EGFR transactivation contributes to the biological activity of FPR in tumor cells. EGFR is a transmembrane cell surface receptor and a member of the c-erb-B family of tyrosine kinases, known to be overexpressed in a variety of human malignant tumors, including carcinomas of the colon, lung, breast, and head and neck (17–20). Human glioma cells have also been shown to express EGFR and, in fact, 40% of clinical cases of glioblastomas show EGFR gene amplification (13). Following ligand binding, EGFR undergoes rapid dimerization and activation resulting in tumor cell proliferation, chemotaxis, angiogenesis, and metastatic spread. Therefore, EGFR has been considered as a molecular target for cancer treatment. In support of this notion, treatment of mice bearing human malignant glioma cells with antibody against EGFR or inhibitors of EGFR tyrosine kinase, considerably, albeit incompletely, suppressed tumor growth (21, 22). Our study confirms that EGFR is also expressed by highly malignant human glioblastoma cells and its depletion by siRNA reduced the tumorigenic capacity of the tumor cells. Nevertheless, our study also showed that depletion of EGFR alone was inadequate to completely suppress the tumorigenicity of glioblastoma cells. This
goal was achieved only when both FPR and EGFR were silenced in tumor cells. Besides activation by its cognate ligand EGF, EGFR has been reported to be transactivated by a considerable number of cell surface molecules to convey growth signals (23). For example, receptors for prolactin, growth hormones, and adhesion molecules as well as stress factors such as oxidants and irradiation are reported to transactivate EGFR. Furthermore, some different GPCRs, in addition to FPR shown in this study, are also capable of transactivating EGFR, including the receptors for lysophosphatidic acid (LPA; refs. 24–26), thrombin (27–29), endothelin-1 (30–34), carbachol (35–37), angiotensin (38–40), bombesin (41, 42), and the chemokine SDF1 (CXCL12; ref. 43). Thus, EGFR plays an important role in assisting the transmission of growth signals in cells in response to a great variety of stimuli.

The mechanisms of EGFR transactivation by GPCRs. The mechanistic basis for GPCRs to transactivate EGFR has not been previously well established and varies in different cell types. However, accumulating evidence suggests that GPCR-induced EGFR transactivation may involve either EGFR ligand–dependent and EGFR ligand–independent pathways. Studies with Rat-1 cell lines (44) have revealed that GPCR for LPA “transactivates” EGFR by activating metalloproteinases to induce EGF shedding from pro-HB-EGF on the cell surface, actually resulting in a more direct ligand activation of the EGFR. This pathway has been ruled out in our experiments because neutralization of either EGF or EGFR with antibodies did not prevent EGFR phosphorylation induced by FPR agonist in glioblastoma cells. Therefore, FPR in glioblastoma cells seems to mediate an intracellular trans-signal cascade that phosphorylates and dimerizes EGFR (Supplementary Fig. S2). This mechanism was also shown in studies of COS-7 cells in which the tyrosine kinase Src plays a crucial role in EGFR phosphorylation in response to LPA and α2A-adrenergic GPCRs, or by overexpression of Gβγ subunits, without involvement of the intrinsic kinase activity of EGFR, which presumably is activated mainly by its cognate ligand, or induction of EGF shedding (45). Our study also showed that whereas Goi was essential for FPR transactivation of EGFR in glioblastoma cells, Src seems to play a key role in linking FPR to the intracellular domains of EGFR where Tyr992 is phosphorylated (Supplementary Fig. S2). Our study additionally revealed that FPR agonist fMLF rapidly induced the dimerization of EGFR in glioblastoma cells, consistent with the observation with the muscarinic acetylcholine receptor, which, when transfected in human embryonic kidney epithelial cells, induced EGFR dimerization (46).

The biological significance of FPR-mediated EGFR transactivation in glioblastoma cells. The transactivation of EGFR by FPR in glioblastoma cells may have important pathophysiologic implications. Although FPR was originally identified in myeloid cells of the innate immune host defense system, its aberrant expression in glioblastoma cells renders tumor cells capable of responding not only to bacterial chemotactic agonist peptides but also to agonist activity contained in the environment of necrotic tumor cells (10). These are likely to be present in tumor tissues because one of the characteristic features of human glioblastomas is the early appearance of necrotic foci due to rapid progression, and ruptured cells are likely to release FPR agonists such as mitochondria peptides (3). In addition, FPR in glioblastoma cells may also interact with other host-derived agonists such as the neutrophil granule protein cathepsin G, produced under inflammatory conditions (5). FPR in glioblastoma cells, although directly contributing to the tumor cell chemotaxis, growth, and production of the angiogenic VEGF (10), seems also to take the advantage of EGFR and by transactivating EGFR, increases the level of tumor cell responses to FPR agonists. This is best shown by the observations in which knocking down either FPR or EGFR each considerably diminished the tumorigenicity of the tumor cells, whereas concomitant depletion by siRNA of FPR and EGFR completely abolished the tumorigenic capacity of glioblastoma cells in nude mice. Thus, our study proposes that FPR and EGFR cooperate to exacerbate the malignant behavior of human glioblastoma cells and targeting both receptors may yield superior therapeutic effects compared with targeting either one receptor.

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