Kispeptin-10-Induced Signaling of GPR54 Negatively Regulates Chemotactic Responses Mediated by CXCR4: a Potential Mechanism for the Metastasis Suppressor Activity of Kispeptins

Jean-Marc Navenot, 1 Zixuan Wang, 1 Michael Chopin, 1 Nobutaka Fujii, 2 and Stephen C. Peiper 1

1Department of Pathology and Immunotherapy Center, Medical College of Georgia, Augusta, Georgia and 2Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan

Abstract

The product of the KiSS-1 gene is absent or expressed at low level in metastatic melanoma and breast cancer compared with their nonmetastatic counterparts. A polypeptide derived from the KiSS-1 product, designated kisspeptin-10 (Kp-10), activates a receptor coupled to Giq subunits (GPR54 or KiSS-1R). To study the mechanism by which Kp-10 antagonizes metastatic spread, the effect on CXCR4-mediated signaling, which has been shown to direct organ-specific migration of tumor cells, was determined. Kp-10 blocked chemotaxis of tumor cells expressing CXCR4 in response to low and high concentrations of SDF-1/CXCL12 and inhibited mobilization of calcium ions induced by this ligand. Pretreatment with Kp-10 did not induce down-modulation of cell surface CXCR4 expression, reduce affinity for SDF-1/CXCL12, or alter Gαi subunit activation stimulated by this ligand. Although Kp-10 stimulated prolonged phosphorylation of extracellular signal-regulated kinase 1/2, it inhibited the phosphorylation of Akt induced by SDF-1. The ability of Kp-10 to inhibit signaling and chemotaxis induced by SDF-1 indicates that activation of GPR54 signaling may negatively regulate the role of CXCR4 in programming tumor metastasis. (Cancer Res 2005; 65(22): 10450-6)

Introduction

The capacity for metastatic spread is a critical aspect of tumor cell biology that has a profound effect on clinical behavior. There is significant evidence that the metastatic phenotype is a composite effect of multiple mechanisms, including breach of normal architectural boundaries, angiogenesis, directed migration, and target site modification. Expression microarray analysis of high bone metastatic variants biologically selected from a nonmetastatic cell line identified CXCR4 among a cadre of genes that confer the metastatic phenotype (1).

CXCR4, the receptor for the CXC chemokine stromal cell–derived factor 1 (SDF-1/CXCL12), is a G-protein–coupled receptor (GPCR) expressed by a wide spectrum of cells and its physiologic importance in hematopoiesis, development of the vasculature and of the central nervous system has been emphasized by the lethal phenotype of its knockout in mice. Among the candidate genes that were implicated in the metastatic phenotype, expression of CXCR4 alone was found to significantly increase the metastatic behavior, and bone metastasis was further increased by the coordinated expression of other prometastatic genes (1). Common target organs for the metastatic spread of breast cancer secrete SDF-1/CXCL12, including lung, lymph node, liver, and bone marrow. Blockade of this receptor with a monoclonal antibody has been shown to inhibit spread of human breast cancer cells to lungs and regional lymph nodes in a mouse xenograft model (2). Although CXCR4 has been implicated in the pathogenesis of metastatic spread of multiple malignant tumors, the regulation of this mechanism has not yet been elucidated (3). It is unclear whether expression of CXCR4 is sufficient to program migration of tumor cells to target organs that secrete SDF-1/CXCL12, or the sensitivity of this receptor to the chemotactic gradient of ligand may be positively and/or negatively regulated by independent factors.

Genes having a metastasis suppressor function are candidates for the negative regulation of prometastatic mechanisms (4). The KiSS-1 gene was originally identified by its altered expression in metastatic melanoma but not in localized tumors (5). Programming of KiSS-1 expression in human breast carcinoma cell lines decreased metastatic spread in mouse xenograft models (6). The KiSS-1 protein (also known as metastin or kisspeptin) contains 145 residues and multiple shorter products resulting from naturally occurring proteolytic cleavage have been identified. Metastin (45-54) [also known as metastin (112-121) or KiSS-1 (112-121), kisspeptin-10 (Kp-10)] is a 10-residue peptide derived from the product of the KiSS-1 (7–11). Kispeptins bind to the same GPCR (KiSS1-R: hOT7T175, AXOR12, and GPR54; refs. 7–9). Qualitatively, all the different forms of the polypeptide (natural or synthetic) have a similar activity but different affinities for their receptor, the decapetide being the most active. Exposure of cancer cell lines (melanoma, pancreatic carcinoma, or Chinese hamster ovary, CHO) with endogenous or programmed expression of GPR54 to metastin decreased expression of metalloproteinase 9, motility, and proliferation in vitro and prevented metastasis in vivo (6–8, 12, 13). Because metastin decreased the motility and migration of cell lines exposed to fetal bovine serum (FBS), it was hypothesized that the antimetastatic action of this ligand may involve negative regulation of prometastatic mechanisms, including directed chemoattraction to target organs. In this study, the effect of Kp-10 on the function of CXCR4, including chemotaxis and intracellular signaling, was determined in CHO and HeLa cell transfectants.

Materials and Methods

Materials. The COOH-terminally amidated decapeptide Kp-10 (YNWNSFGLRF-NH2) was synthesized at the University of Kyoto, Japan and was used in all the experiments. CHO and HeLa were selected because of their total absence of response to Kp-10 as assessed by calcium mobilization and activation of extracellular signal-regulated kinase 1/2 (ERK1/2)/mitogen-activated protein kinase (MAPK). CXCR4 is endogenous
in HeLa and expressed by transfection in CHO. Human GPR54 was subcloned in pcDNA3.1 (Invitrogen, Carlsbad, CA) with a Myc-tag at the NH2 terminus and transfected in both cell lines. Transfectants were selected by magnetic sorting (Miltenyi Biotec, Auburn, CA).

Chemotaxis assay. Cells were resuspended in MEM-α/0.5% bovine serum albumin (BSA) at a density of 2 × 10^5/ml for CHO or 5 × 10^5 for HeLa, and 100 μL were added to the top chamber of a 24-well transwell apparatus (6.5-mm diameter, 80-μm pore size; Corning, New York, NY) coated with collagen (human, type IV, Sigma, St Louis, MO). SDF-1 (Leinco Technologies, St Louis, MO) was added to the lower chamber, and Kp-10 was added either to the bottom chamber or both to the bottom and to the cells in the top chamber. The plates were incubated for 4 hours at 37°C. Cells were fixed with 20% ethanol/0.5% crystal violet, and the cells at the top of the membrane were wiped off. The cells on the bottom face of the membrane were stained with 4,6-diamidino-2-phenylindole and counted with a fluorescence microscope. Alternatively, the cells migrating in the bottom chamber were resuspended in the medium and counted for 1 minute by flow cytometry (LSRII, Becton Dickinson, San Jose, CA) after appropriate gating.

Calcium mobilization. CHO cells were loaded with 2 μg/ml Fura-2 acetoxyethyl ester (Molecular Probes, Eugene, OR). Agonist-dependent increases in cytoplasmic calcium were determined as described (14). Calcium mobilization was also studied by flow cytometry. Cells were then prepared in a similar way except that Indo-1 (Molecular Probes) was used instead of Fura-2. The cells were analyzed on a LSRII flow cytometer equipped with a solid-state UV laser (Cellix, Lightwave Electronics, Mountain View, CA). The fluorescence of Indo-1 was split by a 450-nm LP dichroic mirror and measured by two separate detectors after passage through a 530/30 band pass filter (calcium-free form) and a 405/20 band pass filter (calcium-bound form). Acquisition and analysis of the ratio of fluorescence over time was done with the Diva software (Becton Dickinson). About 300 cells per second were analyzed over the entire experiments.

Flow cytometry. Internalization of CXCR4 was measured by indirect immunofluorescence and flow cytometry. Cells were detached with citrate buffer, resuspended in MEM-α/0.5% BSA at 1 × 10^5 cells/ml. The cells (100 μL) were then exposed to 100 nmol/L of SDF-1 or 100 nmol/L of Kp-10 for 30 minutes at 37°C (or medium alone for the positive control). After an acid wash (pH 3) to elute the ligand from the receptor, cells were incubated with a saturating concentration of a monoclonal antibody to CXCR4 (A145, 10 μg/ml; ref. 15) followed by a phycoerythrin-labeled antibody to mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA).

Ligand binding and displacement. Membrane preparations of CHO cells stably expressing the GPR54 were incubated with 0.1 nmol/L [35S]metastin (45-54) (Amersham Biosciences, Piscataway, NJ) in the presence of incremental concentrations of Kp-10 as described previously (8). The affinity was calculated using Prism (GraphPad Software, San Diego, CA) and is expressed as the EC50 ± SD based on duplicate samples of each concentration.

γ[35S]GTP binding assay. The γ[35S]GTP binding assay was carried out as described previously (14). The effect of Kp-10 on the capacity of CXCR4 to activate G-proteins was measured either by incubating the membrane fraction with Kp-10 immediately before adding SDF-1 or following preincubation of the cells with Kp-10 for 5 minutes at 37°C to allow cross-desensitization before preparation of the membranes.

Figure 1. Kp-10 inhibits chemotaxis mediated by SDF-1 in CHO and HeLa cells expressing CXCR4 and the GPR54. A, Kp-10 inhibits chemotaxis toward 50 nmol/L of SDF-1 in a dose-dependent manner. The reduction of the number of migrating CHO cells depends on the concentration of Kp-10 (P < 0.05 at 1 nmol/L, P < 0.001 at 10 and 100 nmol/L), and the effect is independent of location in the top or the bottom chamber. Results are based on the number of cells counted in 8 high power fields (HPF, ×40) for each condition. B, the inhibitory effect of Kp-10 on CXCR4 requires the presence of GPR54 in CHO cells. Parallel experiments were done in triplicate in CXCR4 transfectants or CXCR4/GPR54 double transfectants. The cells in the bottom chamber of the transwell were counted for 1 minute by flow cytometry. Almost no cells could be counted when GPR54 transfectants were exposed to Kp-10. C, inhibition of chemotaxis toward increasing doses of SDF-1 as well as chemokinesis is also observed in HeLa cells transfected with GPR54. Results are based on the number of cells counted in 8 high power fields and are representative of three independent experiments. D, Kp-10 does not inhibit chemotaxis or chemokinesis of HeLa cells that do not express the GPR54.
Western blot. CHO cells were seeded in 60-mm dishes (5 × 10^5), grown for 24 hours in MEM-α/10% FBS, and starved overnight in DMEM/0.5% BSA. Stimulation of the cells with 100 nmol/L of SDF-1 or Kp-10 or both was done at 37°C. The cells were then washed with ice-cold PBS and resuspended in lysis buffer (50 mmol/L Tris, 10 mmol/L EDTA, 150 mmol/L NaCl, 1% Triton X-100, 0.1% SDS, protease, and phosphatase inhibitors) for 1 hour. After centrifugation (15 minutes at 13,000 rpm), the soluble fraction was diluted in SDS sample buffer. SDS-PAGE and transfer to polyvinylidene difluoride were done according to standard protocols. Antibodies to p44/42 MAPK (ERK1/2), phospho-p44/42, Akt, phospho-Akt Ser473, and phospho-Akt Thr308 (Cell Signaling Technology, Beverly, MA) were detected using horseradish peroxidase–labeled secondary antibodies (Jackson ImmunoResearch Laboratories) and Enhanced Chemiluminescence-Plus (Amersham Biosciences).

Statistics. When relevant, quantitative data were analyzed using the Student's t test.

Results
KiSS-1 inhibits the chemotactic response to SDF-1. The findings related to the relative roles of CXCR4 and Kp-10 in cancer cell metastasis suggest that signaling induced by KiSS-1 may antagonize the effects of SDF-1, thereby suppressing the metastatic spread of breast cancer. The ability of Kp-10 to inhibit the directed migration induced by SDF-1 was tested in CHO transfectants expressing GPR54 and CXCR4. As shown in Fig. 1A, exposure of target cells to a gradient of Kp-10 parallel to a chemotactic gradient of SDF-1 inhibited chemotaxis induced by 50 nmol/L SDF-1 in a dose-dependent fashion. A similar suppression of chemotaxis induced by SDF-1 was noted when Kp-10 was placed in the upper chamber of the transwell and mixed with the cells immediately prior the initiation of chemotaxis (Fig. 1A). The presence of high concentration of Kp-10 (100 nmol/L) had no effect on SDF-1-induced chemotaxis of cells expressing CXCR4 but not GPR54 (Fig. 1B). Parallel experiments were done with HeLa cells, which have endogenous CXCR4 expression and were transfected with GPR54. As shown in Fig. 1C, exposure of HeLa-GPR54 transfectants to 100 nmol/L Kp-10 completely inhibited chemotaxis stimulated by SDF-1 at concentrations of 10 and 100 nmol/L. The magnitude of transmigration of cells exposed to Kp-10 was less than basal levels, consistent with the previous finding that kisspeptins decrease basal cytokinesis (8). Like in the case of CHO cells, no effect of Kp-10 on the chemotaxis induced by SDF-1 and CXCR4 was observed in HeLa cells that did not express the GPR54 (Fig. 1D). The transient nature of the suppressive effect on chemotaxis induced by SDF-1 was evident from the significant decrease in inhibition detected when chemotaxis to SDF-1 was initiated 4 hours following addition of Kp-10 (Supplementary Data 1).

Unilateral desensitization of CXCR4 signaling by GPR54 activation. The effect of GPR54 activation on CXCR4 signaling induced by SDF-1 was determined to elucidate potential mechanisms responsible for the suppression of chemotaxis stimulated by the latter ligand. Both Gi-coupled CXCR4 (and other chemotactic GPCRs) and Gq-coupled GPR54 can mobilize intracellular calcium by activating phospholipase C-β, either directly through the α subunit of Gq (GPR54) or through the Gi/γ heterodimer (CXCR4). Crosstalk between GPCR can occur

Figure 2. Kp-10 inhibits the cytosolic calcium signaling response induced by the stimulation of CXCR4 by SDF-1 in CHO cells. Transfectants of CHO cells expressing CXCR4 and GPR54 were stimulated sequentially by SDF-1 or Kp-10. The addition of the first and the second ligand (arrow) are 3 minutes apart. A, cells stimulated with 100 nmol/L of SDF-1 first then 100 nmol/L of Kp-10 can fully respond to each ligand. B–E, the cells were exposed to either HBSS (B) or 1 mmol/L Kp-10 (C), 10 mmol/L Kp-10 (D), or 100 mmol/L Kp-10 (E; first arrow) followed by 100 nmol/L of SDF-1 (second arrow). The response to SDF-1 was almost completely abolished after exposure to 1 mmol/L of Kp-10 and was undetectable with higher concentrations. Representative of at least three independent experiments. Similar results were obtained in HeLa-GPR54 cells.
suggest a rapid and profound cross-desensitization of CXCR4 by the signaling of the GPR54. Because the activation of GPR54 by Kp-10 generated a slow and delayed increased in cytoplasmic calcium (probably related to calcium influx), a very small response to SDF-1 could be masked and cannot be completely excluded. Wash-out experiments were done in which cells were exposed for 3 minutes to 100 nmol/L of Kp-10, washed thrice, and allowed to recover for various periods of time before being challenged with SDF-1. The recovery of the response to SDF-1 was partial 15 minutes after removal of Kp-10 and complete after 30 minutes (data not shown), indicating that the effect is reversible and requires continuous presence of Kp-10. Exposure of target cells lacking GPR54 expression to Kp-10 induced no calcium mobilization and no cross-desensitization of CXCR4 signaling (data not shown). The specificity of this cross-desensitization was further shown by pretreating the cells with 5-hydroxytryptamine, which has an endogenously expressed Gi-coupled receptor in CHO cells. The stimulation with 5-hydroxytryptamine induced a calcium flux that did not modify the amplitude of the subsequent response to SDF-1 (Fig. 2F). This result illustrates the absence of desensitization between the 5-hydroxytryptamine receptor and CXCR4, although the receptors couple to the same G-protein. Because such a profound effect of GPR54 on the signaling of CXCR4 may be surprising, we tested the homogeneity of the cell response to both ligands by flow cytometry. When CHO cells expressing CXCR4 and GPR54 were exposed to Kp-10, all the cells responded by releasing calcium into their cytoplasm (Fig. 3A). Again, subsequent addition of SDF-1 did not have any effect (Fig. 3A). When SDF-1 was added to the cells before Kp-10, all the cells responded to both ligands (Fig. 3B).

Negative regulation of CXCR4 signaling does not alter receptor biology. The negative regulation of GPR54 activation on the induction of signaling by SDF-1 could result from direct effects on CXCR4 impairing its membrane expression, binding to SDF-1, or coupling to G-proteins. The first hypothesis was tested in down-modulation experiments. A late consequence of the activation of a GPCR by its cognate agonist is the phosphorylation of cytoplasmic domains of the receptor by GPCR-regulated kinases (GRK) and protein kinase C. Interaction with β-arrinestins and internalization of the receptor follow resulting in the desensitization of the receptor. In some cases, activation of one receptor can result in the cross-phosphorylation and desensitization of another one. The possibility of such a mechanism was investigated. As shown in Fig. 4A, exposure of CHO-CXCR4/GPR54 transfectants to SDF-1 resulted in rapid down-modulation of CXCR4 levels on the plasma membrane. In contrast, exposure of these cells to Kp-10 did not alter levels of cell surface CXCR4.

Ligand binding experiments were done to establish whether the decreased response of CXCR4 was the result of decreased affinity for SDF-1. As shown in Fig. 4B, pre-exposure of CHO-CXCR4/GPR54 transfectants to Kp-10 did not result in a significant alteration of the affinity of CXCR4 binding to SDF-1.

The earliest event in GPCR signaling is the activation of Gα subunits of heterotrimeric G-proteins. The effect of GPR54 activation on the induction of Gα subunit binding to GTP by SDF-1 activation of CXCR4 was tested using γ[35S]GTP. Exposure of membrane fractions from CHO-CXCR4/GPR54 transfectants to SDF-1 resulted in a significant increase in γ[35S]GTP over basal levels (Fig. 4C). In contrast, incubation with Kp-10 did not increase γ[35S]GTP binding. Preincubation of membrane fractions with Kp-10 before exposure to SDF-1 did not alter γ[35S]GTP binding.
seen with SDF-1 alone. To exclude the possibility that exposure of membrane fractions to Kp-10 would not allow the cross-desensitization mechanism to be preserved, an alternative experiment was also done where whole cells were exposed to Kp-10 before preparation of the membrane fraction and activation by SDF-1 (Fig. 4C).

We finally investigated whether the normal mechanism of desensitization of CXCR4 after exposure to SDF-1, which results in the internalization of the receptor, was preserved when the cells were pre-exposed to Kp-10. As shown in Fig. 5, pretreatment with Kp-10 only partially reduced the ability of SDF-1 to induce the internalization of CXCR4 (50-60% internalization with both ligands versus 75-80% with SDF-1 alone), suggesting that although part of the signaling of CXCR4 is interrupted, the activated conformation of the receptor is still permissive to the interaction with the GRK.

**GPR54 activation inhibits induction of Akt phosphorylation by SDF-1.** CXCR4 signaling has previously been shown to induce activation of both the MAPK pathway and Akt. Time course experiments done both in CHO (Fig. 6A) and HeLa (Fig. 6B) transfectants showed that the activation of MAPK by the GPR54 extended beyond the extinction of its activation by CXCR4. Whereas activation of CXCR4 by SDF-1 resulted in phosphorylation of Akt on Ser473 and Thr308 in CHO, activation of GPR54 by Kp-10 did not (Fig. 6C). Furthermore, the costimulation of the cells with Kp-10 abolished the effect of SDF-1, showing that the Akt pathway is a target of the crosstalk mechanism between the two receptors.

**Discussion**

The growth and metastasis promoting role of CXCR4 has been well documented (3). However, it is unclear whether regulatory
at least five experiments with similar results.

whereas Kp-10 did not phosphorylate Akt. Costimulation of CXCR4 and GPR54 mobilization and Akt phosphorylation. The suppression of Akt GPR54 blocked distal CXCR4 signaling events, including calcium Ser473 and Thr308 of Akt were phosphorylated upon activation by SDF-1, 100 nmol/L of SDF-1, 100 nmol/L of Kp-10, or both. The efficiency of the stimulation of CXCR4 by SDF-1. CHO cells were exposed for 5 minutes to either of GPR54 by Kp-10 inhibits the phosphorylation of Akt induced by the CXCR4. Suggest a slow desensitization of the signaling of GPR54 is maintained for at least 4 hours in contrast to 1 hour for B, 4 hours, whereas the effect of SDF-1 in the same cells cannot be detected and lysates were prepared at the indicated times from 5 minutes to 4 hours. A, long-lasting activation of ERK1/2 MAPK by GPR54 that is clearly detectable at 4 hours, whereas the effect of SDF-1 in the same cells cannot be detected beyond 1 hour. B, similar results were obtained in HeLa cells in which the signaling of GPR54 is maintained for at least 4 hours in contrast to 1 hour for CXCR4. Suggest a slow desensitization of the signaling of GPR54. C, activation of GPR54 by Kp-10 inhibits the phosphorylation of Akt induced by the stimulation of CXCR4 by SDF-1. CHO cells were exposed for 5 minutes to either 100 nmol/L of SDF-1, 100 nmol/L of Kp-10, or both. The efficiency of the stimulation by each ligand was evaluated by detection of phospho-ERK1/2. Both Ser473 and Thr308 of Akt were phosphorylated upon activation by SDF-1, whereas Kp-10 did not phosphorylate Akt. Costimulation of CXCR4 and GPR54 resulted in the abortion of the activating effect of SDF-1. Representative of at least five experiments with similar results.

(potentiating or inhibitory) mechanisms exist besides the expression of the receptor by tumor cells and of its ligand by target organs. We hypothesized that an antagonism between the metastasis suppressor receptor GPR54 and CXCR4 within tumor cells could modulate the metastatic potential of CXCR4. Here, we show that Kp-10 and its receptor negatively regulate the chemotactic activity of CXCR4. The effect is immediate and sustained but does not influence levels of CXCR4 expression on the cell surface, binding to ligand, or activation of Gα subunits. GPR54 blocked distal CXCR4 signaling events, including calcium mobilization and Akt phosphorylation. The suppression of Akt phosphorylation by Kp-10 mimics the effects of phosphatidylinositol 3-kinase (PI3K) inhibitors, which also block chemotaxis. This raises the possibility that there is equilibrium between signaling pathways of metastasis promoting and suppressor genes through reciprocal crosstalk.

KISS-1 is one of 14 genes that have been shown to suppress metastasis of malignant cells and the only one to bind a GPCR (4). Activation of the GPR54 has been shown to have a variety of effects on tumor cell biology, including suppression of motility induced by FBS, culture scratch repair, proliferation, metastasis of B16 melanoma cells, and invasion in vitro. However, the precise mechanism for the antimetastatic function of Kp-10 is unclear.

PI3K and its downstream effector Akt/protein kinase B can be activated by tyrosine kinase receptors. Multiple GPCRs have also been reported to modulate the activity of Akt, although the exact mechanism is not completely understood. The polarized activation of PI3K and Akt at the leading edge of migrating cells has been described as a key event in chemotaxis (17, 18). If Gi-coupled receptors are generally recognized as activators of PI3K and Akt, the effect of Gq-coupled receptors is more controversial. Several Gq-coupled receptors activate PI3K and Akt, which can result in inhibition of apoptosis (19–22). For instance, it was shown that the activation of Gq-coupled M1, M3, and M5 muscarinic receptors but not Gi-coupled M2 and M4 receptors could protect cells from apoptosis induced by etoposide or UV (20, 23, 24). Other reports suggest instead that signaling of Gq, either activated by a GPCR or in the form of a constitutively active mutant (CAM), can inhibit the activation of Akt induced by growth factors and trigger apoptosis (25–29). Additional studies showed an opposite effect for Gq-coupled receptors (phosphorylation of Akt and inhibition of apoptosis) and CAMs of Gq (reduction of phosphorylation of Akt and apoptosis; ref. 30). These apparently contradictory observations may be reconciled by a model in which Gi/γ subunits would activate PI3K and Akt whereas Gq would inhibit them (30, 31), hypothesis supported by the fact that Gq was shown to interact with PI3K (28).

The suppression of calcium release and the inhibition of Akt activation are at least two ways the KiSS-1 receptor uses to prevent the chemotaxis normally induced by the interaction between SDF-1 and CXCR4. Additional possible factors have already been described, like reorganization of actin fibers and focal adhesion (8, 10). We ignore at this time if the phenomenon is specific for some subtypes of PI3K or Akt or what the exact effect is on the complex signaling network around Akt in tumor cells (32). However, because the antibodies against phosphorylated Akt used here react with all three forms of the kinase and because some of the Western blot experiments were done with total SDS cell lysates, it is likely that any subtype of Akt activated by CXCR4 irrespectively of its subcellular localization will be affected by the GPR54. It should also be noted that the inhibitory effect of the GPR54 on CXCR4-mediated chemotaxis noted in CHO and HeLa cells was reproduced in Jurkat cells, again without any effect of the ligand in the absence of the receptor. This rules out the possibility that the effect could only exist in one particular cell type. It is possible however that the amplitude of the effect depends in part on the expression level of GPR54. It is likely that the remarkable efficacy of the KiSS-1 receptor ultimately results from several coordinated impairments of prochemotactic cellular mechanisms. It is interesting to note that the KiSS-1 receptor completely silences CXCR4 without affecting the receptor itself and without employing
the mechanistic pathways of cross-desensitization between GPCRs (i.e., cross-phosphorylation; data not shown) and down-modulation.

Finally, Akt activity being essential to cell survival, it is likely that the inhibition of its phosphorylation by the KiSS-1 receptor would result in apoptosis, either by itself or in conjunction with other proapoptotic signals. The signaling of the GPR54 and other Gq-coupled GPCRs includes both proapoptotic and antiapoptotic effects. Depending on the cellular environment and the subtypes of signaling molecules involved, one or the other may prevail. However, very recently (33), new information showed that the signaling of the GPR54 in breast tumor cells could specifically promote the expression of an array of proapoptotic genes, suggesting that it may cross the line between metastasis suppressors and tumor suppressors.

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