Kaposi’s Sarcoma-associated Herpesvirus G Protein-coupled Receptor Signals through Multiple Pathways in Endothelial Cells*

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Kaposi’s sarcoma-associated herpesvirus (KSHV; human herpesvirus 8) encodes a chemokine-like G protein-coupled receptor (KSHV-GPCR) that is implicated in the pathogenesis of Kaposi’s sarcoma (KS). Since endothelial cells appear to be targets for the virus, we developed an in vitro mouse lung endothelial cell model in which KSHV-GPCR is stably expressed and KSHV-GPCR signaling was studied. In mouse lung endothelial cells: 1) KSHV-GPCR does not exhibit basal signaling through the phosphoinositide-specific phospholipase C pathway but inositol phosphate production is stimulated by growth-related oncogene α (Gro-α) via a pertussis toxin (PTX)-insensitive pathway; 2) KSHV-GPCR signals basally through a PTX-sensitive pathway leading to a lowering of intracellular cAMP level that can be lowered further by Groo and increased by interferon γ-inducible protein 10; 3) KSHV-GPCR stimulates phosphatidylinositol 3-kinase via a PTX-insensitive mechanism; and 4) KSHV-GPCR activates nuclear factor-κB (NF-κB) by a PTX-sensitive Gβγ subunit-mediated pathway. These data show that KSHV-GPCR couples to at least two G proteins and initiates signaling via at least three cascades in endothelial cells thereby increasing the complexity of regulation of endothelial cell function by KSHV-GPCR that may occur during viral infection.

Kaposi’s sarcoma (KS) is the most common AIDS-related malignancy, presenting as a multifocal tumor predominantly in the skin, visceral organs, and lymph nodes. KS lesions are composed of infiltrating inflammatory cells, endothelial cells, and fusiform or spindle-shaped cells; the origin of spindle cells is controversial but they may be derived from endothelial cells. KS-associated herpesvirus (KSHV; human herpesvirus type 8) is a γ2-herpesvirus that is likely to be the etiological agent of KS (1–4). This virus is present in primary effusion lymphomas and in a subset of cases of multicentric Castleman’s disease. The pathogenic mechanisms by which infection with KSHV leads to disease are still unclear. Among several potential oncopgenes present in its genome, a chemokine-like G protein-coupled receptor, KSHV-GPCR, is a strong candidate to explain KS-mediated pathogenesis. This GPCR exhibits basal signaling (constitutive activity) through the phosphoinositide-specific phospholipase C-InsP pathway in human embryonic kidney and monkey kidney (COS-1) cells (5, 6). KSHV-GPCR expression stimulates proliferation and induces transformation of rodent fibroblasts, and promotes vascular endothelial growth factor-mediated angiogenesis (5, 7, 8). In humans infected with KSHV, KSHV-GPCR transcripts are expressed in KS lesions, specifically in endothelial and spindle-shaped cells, and in tumor cells from primary effusion lymphomas (9–11). KSHV has been proposed from to be causally involved in these malignancies. More recently, transgenic mice expressing KSHV-GPCR under the control of the CD2 promoter/enhancer were reported to develop highly vascularized KS-like tumors, further supporting the contribution of this GPCR to the pathogenesis of KS (12). Since GPCRs in general, and chemokine GPCRs in particular, may exhibit different signaling properties in different cell types, we investigated KSHV-GPCR coupling and signaling in endothelial cells. We chose endothelial cells because they are likely targets of KSHV infection.

The present study characterizes KSHV-GPCR signaling in endothelial cells stably expressing this receptor. We found, as in other cell types, that KSHV-GPCR signals in endothelial cells through a PTX-insensitive, phosphoinositide-specific phospholipase C pathway. We show, for the first time, that in endothelial cells KSHV-GPCR signals also through a separate PTX-sensitive pathway that leads to regulation of intracellular cAMP levels. Last, we confirm that KSHV-GPCR couples to PI3K and to G protein βγ subunit (Gβγ)-mediated activation of NF-κB.

EXPERIMENTAL PROCEDURES

Cell Lines—Microvascular endothelial cells were obtained from lungs of newborn mice carrying an immortalizing transgene composed of DNA for the SV40 large T antigen under the control of regulatory elements of the human vimentin gene (13). Endothelial cells were isolated from minced lung tissue after collagenase digestion and Percoll gradient separation of endothelial cells from other cell types, followed by a differential adhesion procedure to remove residual fibroblasts (14). MECs grew as a monolayer on a gelatin-coated surface, demonstrated contact inhibition at confluence, and were positive for Von Willebrand’s antigen. Proliferation in culture was stimulated by low concentrations of fetal bovine serum (1–5%) and no supplemental growth factors were required. Presence of the transgene renders these endothelial cells immortal. MECs were maintained in DMEM with 5% fetal calf serum (Life Technologies, Inc.). All tissue culture dishes were coated with 0.2% gelatin.

Generation of MLEC Stable Transfectants—MLECs were transfected with either the pcDNA 3.1-KSHV-GPCR (“clone”), pCEFL-KSHV-GPCR (“pool”), or “empty” plasmid (“mock”) (5) using Lipofectin reagent according to the manufacturers recommendations (Life Technologies, Inc.).
Inc.) MLECs (1 × 10^4) were incubated with 2 µg/ml plasmid in serum-free medium. After 5 h, medium containing 5% fetal calf serum and 600 µg/ml active G418 (Geneticin, Life Technologies, Inc.) were added to the cells. In order to obtain clonal transfectants, selected neomycin plates were split to obtain isolated colonies. Colonies and pools (containing multiple clones) were screened for KSHV-GPCRs using specific binding with radiolabeled growth-related oncogene α (Groα), a ligand for KSHV-GPCR (15, 16), and receptor signaling through the inositol phosphate (InsP) pathway. One pool and one clone expressing KSHV-GPCRs (MLEC/KSHV-GPCR), and one pool transfected with empty plasmid (MLEC/mock) were chosen for further studies. Stable transfectants were maintained in G418 and passaged before attaining confluence.

**Competition Binding**—Approximately 70,000 cells were seeded per well in 24-well plates in DMEM with 5% fetal calf serum. Cells were washed with Hank’s balanced salt solution (HBSS, Life Technologies, Inc.) containing 25 mM HEPES, pH 7.4, 125I-Groα (10 µl, PerkinElmer Life Sciences) in the presence or absence of various concentrations of unlabeled Groα were added in ice-cold HBSS supplemented with bovine serum albumin (1 mg/ml), bacitracin (1 mg/ml), and phenylmethylsulfonyl fluoride (1 mM). Plates were incubated at 4°C for 3 h with gentle rocking. Assays were terminated by aspirating the binding buffer, washing the cell monolayers three times with chilled HBSS containing 0.5% NaCl, and solubilizing the cells with 0.4 N NaOH. An aliquot of this cell lysate was added in a 1:10 dilution in 100 µl of luciferase assay buffer and counted on a lysis buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5 mM EDTA and a mixture of protease inhibitors (Sigma). Cells were then scraped and total lysates were incubated end over end at 4°C for 30 min. An aliquot of 15 µl of this lysate was used to assay PI4K activity separately. For PI3K assays, samples were spun down at 500 × g at 4°C and clear lysates were then incubated with 5 µg of PY20 anti-phosphotyrosine antibody (Transduction Laboratories, Lexington, KY) for 1 h with gentle agitation at 4°C. After this step, 50 µl of protein A-Sepharose beads (Sigma) were added to samples for 30 min with gentle agitation at 4°C. Complexes formed with protein A were washed once with lysis buffer and twice with a kinase buffer containing 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.5 mM EDTA and protease inhibitors. Assays were terminated in a 1:1 dilution in 100 µl of kinase buffer containing 10 µg of phosphatidylinositol (Sigma) and incubated 10 min at room temperature before adding 0.02 mg MgCl₂ and 10 µCi of [γ-32P]ATP for another 10 min. Reactions were stopped by adding 100 µl of 1 N HCl. Phosphatidylinositol phosphates were extracted with 200 µl of chloroform/methanol (1:1). The chloroform phase was blown completely to dryness before spotting onto thin-layer chromatography plates (Whatman LK6D) in 30 µl of chloroform. Plates were pretreated with 12% potassium oxalate and samples run in a solvent system of chloroform (60): acetone (20): methanol (50): acetic acid (20): H₂O (20). Plates were exposed to film for 12 h before development.

**RESULTS**

Since endothelial cells appear to be targets for KSHV infection, we focused on KSHV-GPCR coupling and signaling in MLECs expressing these receptors stably. Our previous studies of KSHV-GPCRs in model cells showed that KSHV-GPCR signals through the InsP pathway via a PTX-sensitive pathway. However, GPCRs may exhibit different signaling properties depending on the cell type in which they are expressed. For example, Montaner et al. (see “Discussion”) recently reported that KSHV-GPCR couples to the activation of Akt/protein kinase B via a PTX-sensitive pathway in human umbilical vein endothelial cells (17). We established stable MLECs expressing KSHV-GPCRs and chose to study both a high expressing clone and a pool of G418-resistant cells so as to ensure that the findings were not artifacts of a single clonal phenotype. Binding using 125I-Groα was performed to select these cell populations. As shown in Fig. 1A, specific 125I-Groα binding was observed in the clone and the pool of cells expressing KSHV-GPCRs, whereas there was no specific binding in MLEC/mock. The calculated number of receptors expressed at the cell surface was higher for the clone compared with the pool, 112,000 sites per cell versus 82,000 sites per cell, respectively. We found that the affinity of labeled Groα for the receptor in both cell populations was similar to that found previously (inhibitory constant of −0.2 nM) in other cell types (18, 19).

We tested KSHV-GPCR signaling via the InsP pathway in these cells. As shown in Fig. 1B, there was no measurable production of InsPs in MLEC/mock under basal conditions nor when cells were incubated with Groα. In contrast to findings in other cell types (5, 15), we were unable to detect agonist-independent InsP production in either population of KSHV-GPCR-expressing MLECs. However, InsP production was increased in response to Groα in both MLEC–KSHV-GPCR/pool and MLEC–KSHV-GPCR/clone with a greater increase in the clonal cells. We also tested the inhibition of Groα-stimulated
InsP production by IP-10, which was shown previously to be an inverse agonist for KSHV-GPCR (18). The results presented in Fig. 1 show that IP-10 inhibited Gro/H9251-stimulated InsP production in the MLEC/KSHV-GPCR/clone with a half-maximal inhibitory concentration of 1.4 nM. Similar results were obtained with the MLEC/KSHV-GPCR/pool (data not shown). Thus, KSHV-GPCR expressed in MLECs displayed pharmacological characteristics, namely Gro/H9251 as an agonist and IP-10 as an antagonist, consistent with those reported previously in other cell systems. There was no evidence of basal (or agonist-independent) InsP production in MLECs expressing KSHV-GPCRs.

To gain insight into which G protein(s) couple(s) KSHV-GPCR to the InsP pathway in endothelial cells, we studied the effect of PTX. In all other cell types studied, KSHV-GPCR stimulation of InsP production was not affected by PTX treatment and it was concluded that KSHV-GPCR may couple to a member of the Gq subfamily of G proteins because coupling to Gi/o would be inhibited by PTX (5, 7). However, in the MLEC/KSHV-GPCR/clone, we unexpectedly found that PTX pretreatment increased InsP production stimulated by Gro/H9251 (Fig. 2). Similar results were found for MLEC/KSHV-GPCR/pool (data not shown). Since we did not find that PTX inhibited Gro/H9251-stimulated InsP production, we concluded that phosphoinositide hydrolysis was not mediated by Gi/o. It was possible, however, that the enhancing effect of PTX pretreatment on InsP production stimulated via KSHV-GPCRs was secondary to an increase in cellular cAMP since PTX would uncouple inhibition of adenylyl cyclase caused by Gi/o. We, therefore, measured the effect of PTX pretreatment on cAMP production in these cells. As shown in Fig. 3, PTX had no effect on cAMP production in MLEC/mock. In contrast, cAMP levels were increased by PTX in both MLEC/KSHV-GPCR/pool and MLEC/KSHV-GPCR/clone. It was possible, therefore, that the effect of PTX to enhance KSHV-GPCR-stimulated InsP production was caused by increased cAMP. To test this hypothesis, we measured Groa stimulation of InsP production in KSHV-GPCR-expressing cells incubated with forskolin, a direct activator of adenylyl
cAMP levels in these cells (see below). Fig. 4 illustrates that forskolin treatment of the cells had no effect on Groα-stimulated InsP production in the MLEC/KSHV-GPCR/clone. Similar results were observed in MLEC/KSHV-GPCR/pool (data not shown). Therefore, cAMP was not the mediator by which PTX increased InsP production via KSHV-GPCR.

Because PTX acts to uncouple GPCRs from activating Gαo, the observation that PTX increased cAMP production in KSHV-GPCR-expressing endothelial cells (Fig. 3) is consistent with the idea that chronic inhibition of adenylyl cyclase activity may occur by a receptor-mediated mechanism in MLEC/KSHV-GPCRs. However, the identity of the responsible receptor was not clear. To determine whether the persistent inhibition of cAMP production resulted from KSHV-GPCR signaling, we studied the effects of Groα and IP-10 on forskolin-stimulated cAMP production in MLEC/KSHV-GPCR/clone (Fig. 4A). The data show that Groα, an agonist of KSHV-GPCR, lowered cAMP levels whereas IP-10, a negative antagonist (inverse agonist) of KSHV-GPCR, increased cAMP levels. These data offer pharmacological evidence that basal and Groα-stimulated KSHV-GPCR signaling inhibits cAMP production and, moreover, are consistent with the idea that KSHV-GPCR couples to Gαo in these endothelial cells.

To support this idea further, we measured the effects of PTX on Groα lowering of forskolin-stimulated cAMP levels. As shown in Fig. 5B, the Groα inhibitory effect in MLEC/KSHV-GPCR/pool was partially prevented by PTX treatment. Similar results were obtained with MLEC/KSHV-GPCR/clone (data not shown). Neither PTX nor Groα altered forskolin-stimulated cAMP production in MLEC/mock cells (data not shown). Thus, Groα inhibition of forskolin-stimulated cAMP production by KSHV-GPCR occurs via coupling to PTX-sensitive Gαo proteins to inhibit adenylyl cyclase, Gβ may also serve as an effector (20). To determine whether Gα may mediate signaling after activation of KSHV-GPCR, we measured the effects of KSHV-GPCR signaling on activation of NF-κB activity, which may be stimulated by Gβ (21). Fig. 7 illustrates NF-κB activity in MLEC/KSHV-GPCRs. To determine if NF-κB was activated specifically by KSHV-GPCR, we measured the effect of Groα stimulation, using two different concentrations (10 nM, which is half-maximally effective, and 100 nM, which near-maximally effective) and the effect of IP-10 (Fig. 7, NF-κB bars). NF-κB activity was increased 2-fold over basal by 100 nM Groα and by 1.4-fold by 10 nM Groα. No change in NF-κB activity was observed in MLEC/mock in any condition investigated (data not shown). IP-10 lowered NF-κB activity by 20% thereby providing evidence of basal signaling of the receptor in these cells via a NF-κB-mediated pathway.

To gain insight into the mechanism of KSHV-GPCR activation of NF-κB, we assessed the effects of PTX treatment (Fig. 7, NF-κB + PTX bars). PTX inhibited Groα-stimulated NF-κB activity and abrogated the inhibition caused by IP-10. Therefore, KSHV-GPCR regulation of NF-κB activity appeared to be mediated by Gαo. To determine whether the specific mediator was Gαo or Gβγ, we transfected MLEC-KSHV-GPCR cells with Ga transducin, which acts as a scavenger to sequester Gβγ and would prevent released Gβγ from activating NF-κB. As shown in Fig. 7 (NF-κB + GaT bars), Ga transducin expression in MLEC-KSHV-GPCR cells inhibited NF-κB activation. This inhibition was comparable to that induced by PTX treatment alone. With 100 nM Groα, PTX and Ga transducin expression inhibited NF-κB activity to similar extents (by 65 to 70%). We also exposed cells expressing Ga transducin to PTX (Fig. 7, NF-κB + GaT + PTX bars) and observed that the inhibition of Groα stimulation of NF-κB appeared to be addi-
tive. However, although the effects of PTX and expression of Gα transducin appeared to be additive, to be certain that a Gα/H9251/H9252/H9253-independent mechanism was involved it would be necessary to ensure that both PTX and Gα transducin were maximally effective under the experimental conditions used. These data provide evidence that KSHV-GPCR couples to PTX-sensitive Gi/o protein leading to activation of NF-κB primarily by Gα/H9252/H9253 subunits.

DISCUSSION

Seven years after its discovery (22), a consensus has developed that KSHV is the etiological agent of KS. The virus is consistently found in KS lesions, and is located principally in microvascular endothelial cells and the spindle tumor cells (KS cells) (11, 23, 24). KSHV belongs to a family of transforming viruses and is believed to contain more potential oncogenes in its genome than the other members. Among these transforming genes, ORF 74 encoding a G protein-coupled receptor has been shown to be a good candidate to explain KS pathogenesis, at least in part (25). Although it has not been proved that KSHV-GPCRs are expressed in KS lesions in a functional form, evidence has been provided that expression of signaling KSHV-GPCRs can lead to tumorigenesis in models in which cells expressing KSHV-GPCRs are transplanted into mice (7) and in transgenic mice expressing KSHV-GPCR under the control of CD2 promoter/enhancer (12). These tumors are angioproliferative lesions resembling KS, including features of KS-like spindle cells. Since KS-spindle cells are believed to be of endothelial origin, and are target cells for the virus, we developed an in vitro model in which KSHV-GPCR is stably expressed in mouse lung endothelial cells and tested receptor function in these transfected endothelial cells. Until recently, studies of KSHV-GPCR signaling have been conducted with rat fibroblasts, monkey kidney cells, and human embryonic kidney (HEK293) cells. These cell types, although very useful, give only a limited picture of KSHV-GPCR biology. A report published as our studies were being completed described effects of transient expression of KSHV-GPCRs in human umbilical vein endothelial cells (17) (see below).

KSHV-GPCR was previously shown to exhibit high ligand-independent signaling via a PTX-insensitive phospholipase C pathway (5, 15). Since signaling properties of GPCRs may differ in different cell types, we analyzed KSHV-GPCR signaling in mouse lung endothelial cells. We constructed cell lines stably expressing KSHV-GPCR/clone cells so as to be able to measure endogenous signaling cascades in cell populations. KSHV-GPCRs expressed stably in MLEC/KSHV-GPCR clone cells were transfected with PTX for 4 h prior to addition of 10 μM forskolin and 100 nM Groα or 100 nM IP-10 and measurement of cAMP. The data represent the mean ± S.D. of triplicate determinations in one representative experiment out of three separate experiments.

FIG. 5. Effects of Groα, IP-10, and PTX on forskolin-stimulated cAMP production in MLEC/KSHV-GPCR cells. A, effects of Groα and IP-10 on forskolin-stimulated cAMP production in MLEC/KSHV-GPCR/clone cells. Cells were incubated with 10 μM forskolin and increasing concentrations of Groα or IP-10. The data represent the mean ± S.D. of triplicate determinations in one representative experiment out of three separate experiments. B, effect of PTX on Groα inhibition of forskolin-stimulated cAMP production in MLEC/KSHV-GPCR/pool cells. Cells were incubated with 1 μg/ml PTX for 4 h prior to addition of 10 μM forskolin and 100 nM Groα or 100 nM IP-10 and measurement of cAMP. The data represent the mean ± S.D. of triplicate determinations in one representative experiment out of three separate experiments.

FIG. 6. Effect of KSHV-GPCR expression on PI3K activity in MLECs. A, PI3K and PI4K activities were measured in MLEC/mock and MLEC/KSHV-GPCR clone cells as described under “Experimental Procedures.” B, MLEC/mock and MLEC/KSHV-GPCR clone cells were transfected with PTX for 4 h prior to measurement of PI3K activities.
Fig. 7. Effects PTX and expression of Gα transducin on KSHV-GPCR-mediated activation of NF-κB in MLEC/KSHV-GPCR/clone cells. Cells were incubated with 1 μg/ml PTX for 4 h prior to addition of 10 or 100 nM Groc or 100 nM IP-10. Luciferase activity was measured after an additional 24 h. The data represent the mean ± S.D. of triplicate determinations in one representative experiment out of three separate experiments.

and signaled through a phospholipase C pathway that was not inhibited by PTX (see below). However, we observed that although Groc stimulated increased InsP production and Groc-stimulated InsP production was inhibited by IP-10, as reported in other cell types (5, 18), there was no measurable basal signaling via this pathway in MLECs expressing KSHV-GPCRs. This first major difference compared with previous reports led us to look for basal signaling activity by KSHV-GPCR via other pathways. Indeed, we provide strong evidence that in endothelial cells KSHV-GPCR constitutively inhibits cAMP production and lowers basal cellular cAMP levels. This conclusion is most directly supported by the findings that the KSHV-GPCR-specific inverse agonist IP-10 increases cAMP levels in MLEC/KSHV-GPCR cells (Fig. 5A). In previous reports, it was concluded that KSHV-GPCR signaling was coupled only to a Gq protein family member since InsP production was not affected by PTX treatment. In our study, we showed that KSHV-GPCR couples to a PTX-sensitive Gi/o protein that causes lowering of cAMP levels and activation of the transcription factor NF-κB, a common pleiotropic mediator involved in many biological processes including oncogenesis, control of apoptosis, differentiation, and cell migration. These effects of KSHV-GPCR on cAMP have not been previously reported but are similar to the signaling effects of mammalian chemokine receptors (26).

As noted above, Montaner and colleagues (17) recently reported on their studies of KSHV-GPCR signaling in endothelial cells. In contrast to our use of mouse endothelial cells stably expressing KSHV-GPCRs, they used human umbilical vein endothelial cells that transiently expressed KSHV-GPCRs. Montaner et al. (17) found that KSHV-GPCR expression caused an increase in cell survival that was mediated by Akt/protein kinase B and deduced that KSHV-GPCR activated PI3K because wortmannin inhibited activation of exogenously expressed Akt. We confirmed their finding that KSHV-GPCR increases survival of endothelial cells (data not shown) and showed that KSHV-GPCR signals through the PI3K/Akt pathway by measuring endogenous PI3K activity directly. In addition, we studied whether cAMP might mediate the effects of KSHV-GPCR on PI3K activity. We found that increases in intracellular cAMP stimulated with forskolin had no effect on PI3K activity. Thus, neither the increase in PI3K activity nor in the production of InsP second messengers stimulated by KSHV-GPCR is mediated by cAMP. Both our study and that of Montaner et al. (17) show that in endothelial cells KSHV-GPCR activates the transcription factor NF-κB, signals via PTX-sensitive as well as PTX-insensitive pathways, and that some of the effects of KSHV-GPCR are mediated by Gβγ subunits. Thus, it may be concluded that KSHV-GPCR signals through several independent pathways in endothelial cells and that there are likely to be many diverse biological effects associated with expression of KSHV-GPCRs in these cells.

An unexpected finding was that PTX treatment enhanced Groc-stimulated InsP production in MLEC-KSHV-GPCR cells. This observation was unexpected because in other cells PTX had no effect on KSHV-GPCR-stimulated InsP production and for several GPCRs, including mammalian chemokine receptors (26), PTX diminishes InsP production because these receptors are coupled to activation of phospholipase C by Gi/o proteins. It appears, therefore, that in MLECs, as in other cell types, KSHV-GPCR stimulation of InsP production is primarily mediated by Gi family members. The mechanism whereby PTX led to an increase in KSHV-GPCR-stimulated InsP production remains unclear. We tested the possibility that the increase in cAMP caused by PTX (Fig. 3) could increase the activity of the phospholipase C that causes hydrolysis of phosphoinositides. However, our data show that increased Groc-stimulated InsP production after PTX treatment was not a response to increased cAMP because elevating cAMP directly with forskolin did not affect InsP production. Another possibility was that PTX uncoupled a receptor that inhibits InsP production. However, no GPCR has been identified that directly couples via a G protein to inhibit phospholipase C, as GPCRs couple via Gi proteins to inhibit adenylyl cyclase (20). Some GPCRs that couple via PTX-sensitive G proteins, such as receptors for somatostatin and dopamine, have been found indirectly to inhibit phospholipase C activity by causing a lowering of cytoplasmic free calcium ion concentration (27); it is possible that this occurred in KSHV-GPCR-expressing MLECs. Indeed, it remains possible that KSHV-GPCR could lower cytoplasmic calcium ion concentration via coupling to Gi proteins and that PTX reversed this effect on calcium thus permitting increased phospholipase C activity and increased phosphoinositide hydrolysis. This possibility could be tested in future studies.

In conclusion, this study shows that KSHV-GPCRs couple to multiple pathways in endothelial cells including the adenylyl cyclase-cAMP pathway. These findings confirm and extend previous observations that both PTX-sensitive and PTX-insensitive pathways are involved in KSHV-GPCR signaling and that both Go and Gβγ subunits serve as effectors for KSHV-GPCR signaling. These findings are consistent with the concept that KSHV-GPCR may regulate diverse processes in endothelial cells that are important for KS viral survival and that may lead to human disease.

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