

The Kaposi's Sarcoma–Associated Herpesvirus G Protein–Coupled Receptor as a Therapeutic Target for the Treatment of Kaposi's Sarcoma

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Abstract

The Kaposi's sarcoma–associated herpesvirus (KSHV) encodes a G protein–coupled receptor (vGPCR) that has been implicated in the initiation of Kaposi's sarcoma, identifying vGPCR as an attractive target for preventing Kaposi's sarcoma. However, as only a fraction of cells in advanced Kaposi's sarcoma lesions express vGPCR, it is unclear whether this unique viral oncogene contributes to Kaposi's sarcoma progression. We therefore set out to determine whether the few cells that express vGPCR in established tumors represent an appropriate therapeutic target for the treatment of patients with preexisting Kaposi's sarcoma. To this end, we generated endothelial cell lines stably expressing vGPCR or key KSHV latently expressed proteins (vCyclin, vFlip, and LANA1). The endothelial cell line expressing vGPCR was rendered sensitive to treatment with the nucleoside analogue ganciclovir by using a bicistronic construct coexpressing the herpes simplex virus 1 thymidine kinase. *S.c.* injection into nude mice with mixed-cell populations formed tumors that approximate the ratio of vGPCR-expressing and KSHV latent gene-expressing cells. These mice were then treated with ganciclovir to specifically target only the vGPCR-expressing cells. Surprisingly, despite the expression of KSHV latent genes in the vast majority of tumor cells, specifically targeting only the few vGPCR-expressing cells in established tumors resulted in tumor regression. Moreover, we observed an increase in apoptosis of latent gene-expressing cells after the pharmacologic deletion of the vGPCR-expressing cells. These findings indicate that vGPCR may play a key role in Kaposi's sarcoma progression and provide experimental justification for developing molecular-based therapies specifically targeting vGPCR and its effectors for the treatment of Kaposi's sarcoma patients. (Cancer Res 2006; 66(1): 168-74)

Introduction

The Kaposi's sarcoma–associated herpesvirus (KSHV or human herpesvirus-8), the etiologic agent for Kaposi's sarcoma (1), encodes an arsenal of putative oncogenes that harbor transforming potential *in vitro* (2). Several of these candidate oncogenes are latent genes, expressed in the majority of Kaposi's sarcoma tumor or spindle cells, and are therefore thought to play an important role in Kaposi's

sarcomagenesis. However, accumulating evidence suggests that the expression of latent proteins may not be sufficient to initiate Kaposi's sarcoma. Conversely, expression of the KSHV-encoded viral G protein–coupled receptor (vGPCR) as a transgene or by endothelial-specific retroviral infection is sufficient to induce Kaposi's sarcoma-like tumors in mice (3–5), implicating this viral oncogene in the initiation of Kaposi's sarcoma (6, 7). This suggests that therapies targeting this receptor or its downstream effectors (8) may be an effective approach to prevent the formation of new Kaposi's sarcoma lesions in KSHV-infected patients. However, immunohistochemical examination of biopsies from patients with established Kaposi's sarcoma lesions has revealed that expression of vGPCR is detected in only a fraction of tumor cells (9), raising whether vGPCR would be an appropriate therapeutic target in patients with preexisting Kaposi's sarcoma. Indeed, as the majority of Kaposi's sarcoma tumor cells primarily express latent genes, these viral gene products would be expected to represent more suitable targets for the treatment of Kaposi's sarcoma (10). Unfortunately, due the lack of suitable animal models to study Kaposi's sarcoma promotion *in vivo*, it has been difficult to assess the relative contribution of, and complex interplay among, these genes to Kaposi's sarcomagenesis.

We show here that coinjection of endothelial cells expressing latent genes with a few endothelial cells stably expressing vGPCR—at a ratio that approximates the proportion of vGPCR-expressing and latent gene-expressing tumor cells found in human Kaposi's sarcoma—synergistically enhances latent gene-driven tumorigenicity. Indeed, immunohistochemical analysis of tumors formed using this system revealed that the majority of cells expressed the latent genes, whereas vGPCR is expressed in only rare tumor cells, analogous to human Kaposi's sarcoma. Surprisingly, however, pharmacologic deletion of these rare vGPCR-expressing cells is sufficient to cause tumor regression. We observed an increase in apoptosis of tumor cells as a consequence of the pharmacologic deletion of the vGPCR-expressing cells. Furthermore, although expression of KSHV latent genes was still detected in few surviving cells, these residual cells lost their tumorigenic potential in the absence of the paracrine secretions from vGPCR-expressing cells. Thus, using a Kaposi's sarcoma model system that can recapitulate the complex interplay between lytic and latent infected cells, we provide evidence for the feasibility of specifically targeting vGPCR-expressing cells as a therapeutic approach for the treatment of patients with Kaposi's sarcoma.

Materials and Methods

Expression plasmids and reagents. The expression plasmids for vGPCR, vGPCR (R143A), vCyclin, vFlip, vCyclin/vFlip, Kaposin, vBcl2, vIRF1, vIL6, and enhanced green fluorescent protein have been described

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doi:10.1158/0008-5472.CAN-05-1026

previously (3, 11). The expression plasmid for LANA1 was generously provided by Dr. C. Boshoff (University College of London, London, United Kingdom) and subsequently subcloned into the pCEFL eukaryotic expression vector. The expression plasmid for K1 was generously provided by Dr. J. Jung (Harvard University, Cambridge, MA) and subsequently subcloned into the pCEFL eukaryotic expression vector. The bicistronic construct vGPCR-herpes simplex virus 1 thymidine kinase (HSV1-TK) was obtained by first inserting vGPCR and then HSV1-TK into pCEFL internal ribosome entry site (12). Ganciclovir was purchased from EMD Biosciences (San Diego, CA). For *in vitro* studies, this compound was reconstituted in PBS as a 50 mmol/L stock solution, which was further diluted to the working concentration (0-1,000 nmol/L) in culture medium.

Cell lines and transfections. SV40 large T-antigen immortalized murine endothelial cells (SVEC) were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Transfection was done using Fugene reagent (Roche Applied Science, Indianapolis, IN) according to the manufacturer's protocol. Stable SVEC cell lines were obtained by stable transfection of the corresponding pCEFL-derived plasmids as described previously (3).

ELISA. Conditioned medium from EC-vGPCR, EC-R143A, or SVEC parental cell lines was prepared as described previously (13). Assays for cytokine secretion were done by Pierce Biotechnology (Rockford, IL).

Assessment of [³H]thymidine incorporation. Assessment of cell proliferation by uptake of [³H]thymidine (ICN Pharmaceuticals, Inc., Costa Mesa, CA) was essentially as described previously (13).

Establishment and treatment of tumor allografts in athymic *nu/nu* mice. All animal studies were carried out using the appropriate NIH animal care and user protocol. SVECs (10⁶ cells) stably expressing vGPCR were used to induce allografts in 6-week-old athymic (*nu/nu*) nude female mice as described (3). For drug treatment, tumor-bearing animals were randomly grouped (control, *n* = 10; test, *n* = 10) and treated with ganciclovir (50 mg/kg/d) or an equal volume of diluent (PBS). Treatment schedule was a single injection per animal given *i.p.* for 4 consecutive days. For analysis, tumor weight was determined as described previously (3), whereby tumor volume ($LW^2 / 2$, where *L* and *W* represent the longest length and shortest width of the tumor, respectively) was converted to weight. Results of animal experiments were expressed as mean ± SE. At the end of the study period, animals were euthanized for tissue retrieval, which was fixed (4% paraformaldehyde overnight before processing for paraffin embedding) for immunohistochemical analysis.

Immunohistochemistry. Tissues were fixed in 4% paraformaldehyde-1 × PBS for 36 hours, transferred to 70% ethanol/PBS, and embedded in paraffin. Immunohistochemical analysis of tissues has been described previously (3).

Results

KSHV vGPCR potently renders expressing endothelial cells tumorigenic. To establish a mouse model in which we could study the contribution of candidate KSHV oncogenes to Kaposi's sarcomagenesis, we took advantage of the availability of non-tumorigenic SVECs. When injected *s.c.* into nude mice, 10⁶ SVECs are unable to form tumors up to 6 months after injection (data not shown) but are rendered tumorigenic by stable introduction of an oncogene, enabling rapid *in vivo* screening for tumorigenic proteins. We generated SVEC lines stably expressing key KSHV latent genes *vCyclin* (EC-vCyclin), *vFlip* (EC-vFlip), *LANA1* (EC-LANA1), and *Kaposin* (EC-Kaposin) or lytic genes *K1* (EC-K1), *vBcl2* (EC-vBcl2), *IRF1* (EC-IRF1), *vIL6* (EC-vIL6), or *vGPCR* (EC-vGPCR). We first confirmed the expression of these genes by Western blot analysis (data not shown) before examining their tumorigenic potential *in vivo*. Despite that many of these genes may harbor transforming capability *in vitro* (2), only endothelial cells expressing the lytic

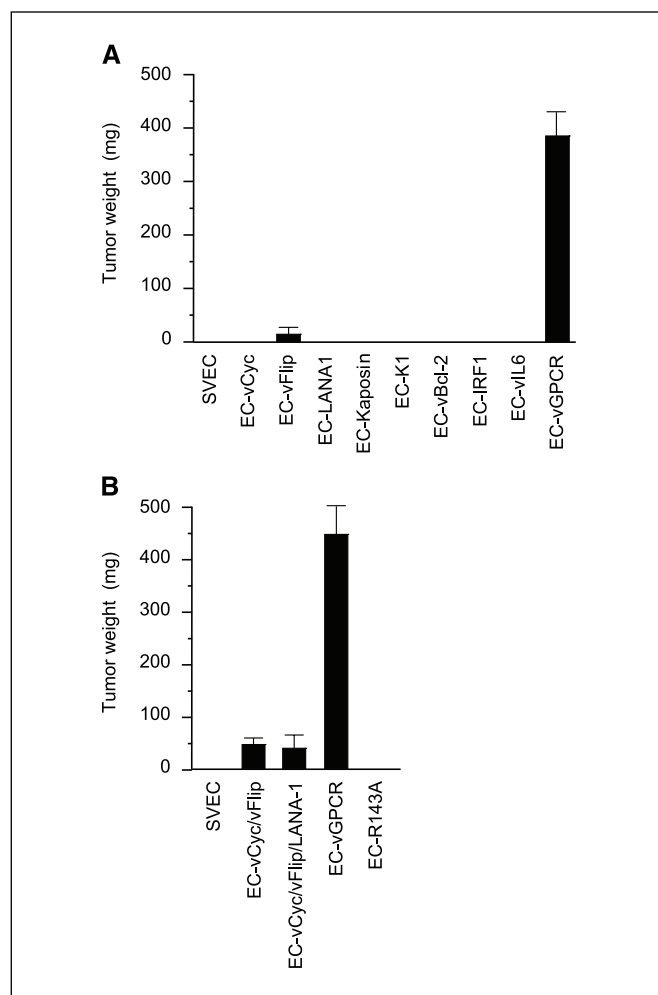


Figure 1. vGPCR is sufficient to render expressing endothelial cells oncogenic. **A**, weight of tumors formed from *s.c.* injection of nude mice with 10⁶ SVECs stably expressing vCyclin (EC-vCyclin), vFlip (EC-vFlip), LANA1 (EC-LANA1), Kaposin (EC-Kaposin), K1 (EC-K1), vBcl2 (EC-vBcl2), IRF1 (EC-IRF1), vIL6 (EC-vIL6), or vGPCR (EC-vGPCR). SVEC parental cells were used as a control. **B**, weight of tumors formed from *s.c.* injection of nude mice with endothelial cell lines stably expressing vCyclin and vFlip (EC-vCyclin/vFlip), vCyclin, vFlip, and LANA1 (EC-vCyclin/vFlip/LANA1), vGPCR (EC-vGPCR), or the inactive vGPCR R143A mutant (EC-R143A). SVEC parental cells were used as a control. Tumor weight was estimated as described in Materials and Methods.

gene, *vGPCR* (EC-vGPCR), consistently formed tumors when injected *s.c.* into nude mice (Fig. 1A). To assess whether latent genes could cooperate in endothelial cell transformation, we also prepared endothelial cell lines stably coexpressing *vCyclin* and *vFlip* (EC-vCyclin/vFlip) or *vCyclin*, *vFlip*, and *LANA1* (EC-vCyclin/vFlip/LANA1). As shown in Fig. 1B, EC-vCyclin/vFlip cells were only weakly tumorigenic. Surprisingly, addition of a third KSHV latent gene, *LANA1* (EC-vCyclin/vFlip/LANA1), failed to further enhance the tumorigenic potential of expressing endothelial cells (Fig. 1B), suggesting that cooperation among latent genes may not be sufficient either to explain the tumorigenic potential of KSHV infected cells. This is in striking contrast to the potent tumorigenic potential of endothelial cells expressing the KSHV lytic gene, *vGPCR* (Fig. 1A and B). Collectively, prior studies (3-7) and these results suggest that vGPCR may be one of the most potent oncogene encoded by KSHV.

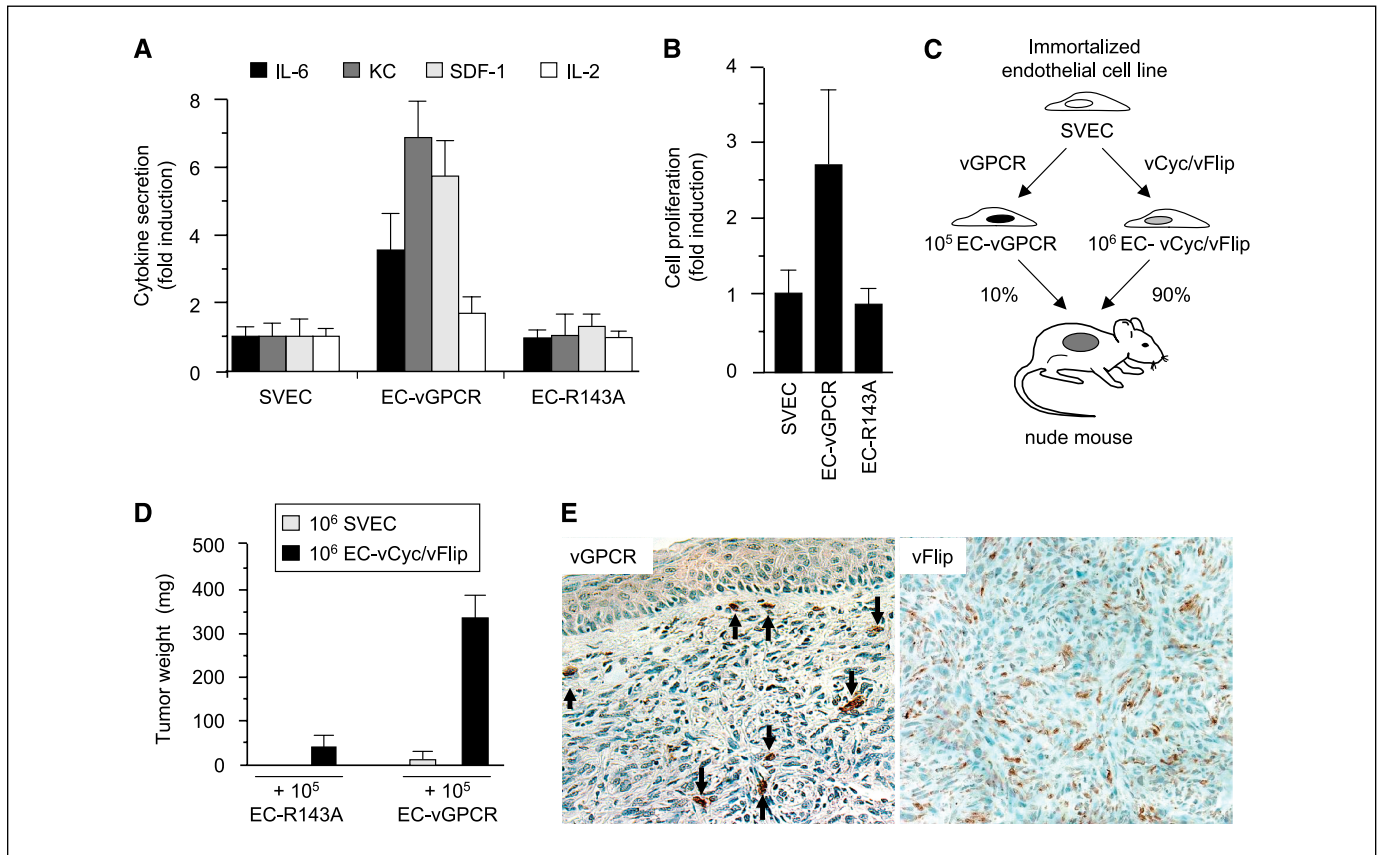


Figure 2. vGPCR promotes the tumorigenic potential of a combination of KSHV latent genes in a mouse allograft model for Kaposi's sarcoma. *A*, secretion of key Kaposi's sarcoma cytokines, IL-6, murine IL-8/Gro- α homologue (KC), SDF-1, and IL-2 by endothelial cells expressing vGPCR (EC-vGPCR) or its inactive mutant, R143A (EC-R143A). *Columns*, mean fold induction with respect to secretion by control (SVEC) cells; *bars*, SD. *B*, proliferation of EC-vCyclin/vFlip treated with conditioned medium of cultured endothelial cells expressing vGPCR (EC-vGPCR) or its inactive mutant, R143A (EC-R143A), determined by the incorporation of [³H]thymidine. *Columns*, mean fold induction with respect to results obtained using conditioned medium from control (SVEC) cells; *bars*, SD. *C*, schematic representation of the Kaposi's sarcoma allograft model in which mixed-cell populations of lytic (vGPCR) and latent gene-expressing endothelial are co-injected into nude mice in a ratio that approximates their expression pattern in human Kaposi's sarcoma. *D*, weight of tumors formed from coinjection with mixed-cell populations of these cell lines. *E*, immunohistochemical analysis of tumors formed using this model system for Kaposi's sarcoma showing rare tumor cells expressing vGPCR (*left*), with the majority of cells expressing latent genes (*right*).

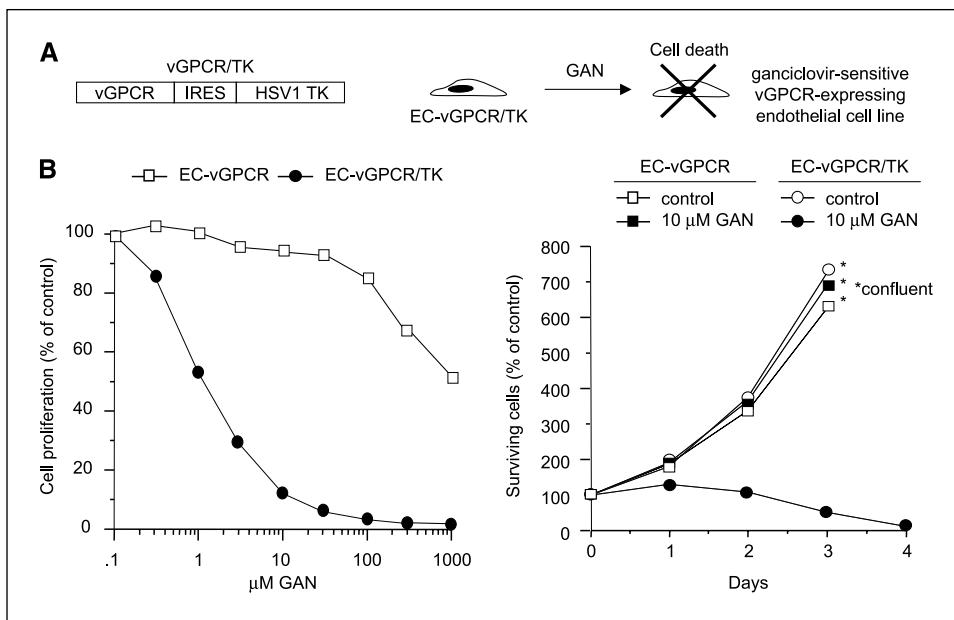


Figure 3. vGPCR-expressing endothelial cells are rendered sensitive to ganciclovir treatment by coexpressing HSV1-TK. *A*, schematic representation of an endothelial cell line stably expressing a bicistronic construct encoding both vGPCR and HSV1-TK (EC-vGPCR/TK), rendering this cell line sensitive to treatment with the nucleoside analogue, ganciclovir (GAN). *B*, increasing doses (0.1-1,000 $\mu\text{mol/L}$) of ganciclovir inhibits cell proliferation of EC-vGPCR/TK but not EC-vGPCR as determined by incorporation of [³H]thymidine. *C*, extended treatment of EC-vGPCR/TK with 10 $\mu\text{mol/L}$ ganciclovir induced cell death in ~100% of treated cells within 4 days. Cells treated with vehicle control are indicated. EC-vGPCR was completely insensitive to this dose of ganciclovir, reaching confluence within 3 days.

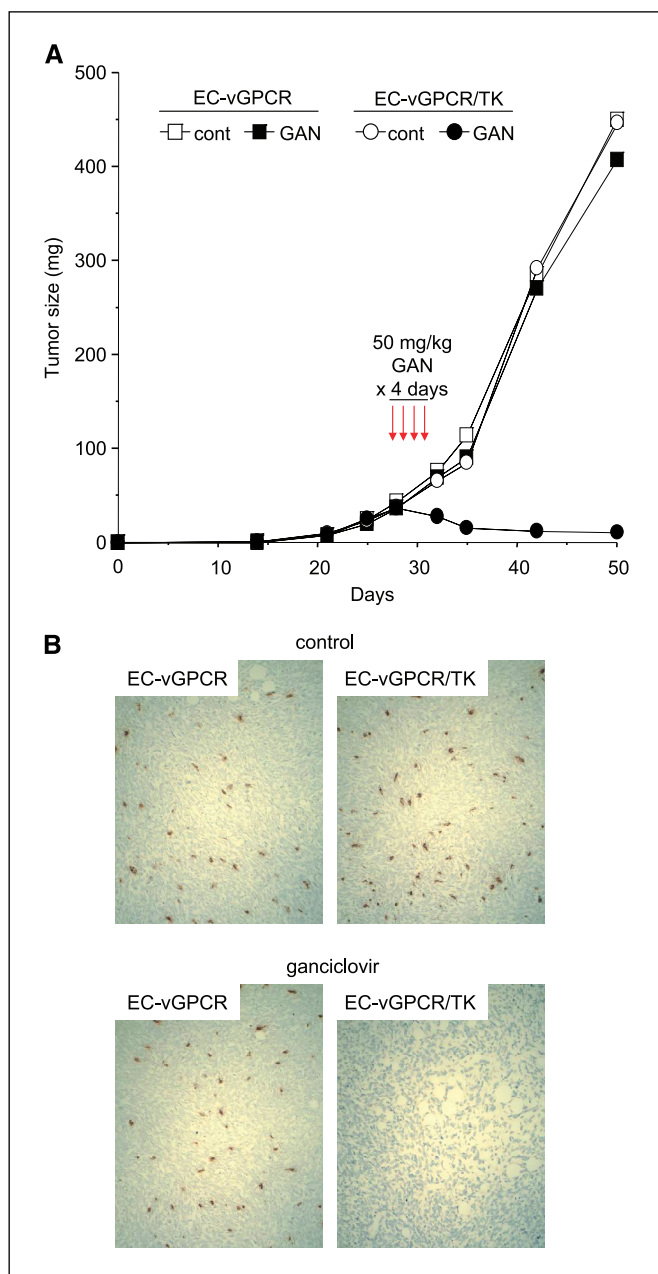


Figure 4. vGPCR/TK-expressing endothelial cells are sensitive to ganciclovir treatment *in vivo*. **A**, established tumors formed from s.c. injection of nude mice with EC-vGPCR or EC-vGPCR/TK were treated with PBS (*cont*) or a tolerable dose of ganciclovir (50 mg/kg) for 4 consecutive days when they reached ~50 mg. Tumor weight was calculated ($LW^2/2$, where L and W represent the longest length and shortest width of the tumor, respectively) and recorded biweekly. **B**, immunohistochemical analysis of EC-vGPCR or EC-vGPCR/TK tumors treated with PBS (*control*) or ganciclovir revealing a complete loss of all vGPCR-expressing cells in EC-vGPCR/TK tumors 24 hours after completion of the 4-day treatment cycle.

A mouse allograft model for Kaposi's sarcoma. vGPCR is a constitutively active GPCR closely related to the mammalian cytokine receptor, CXCR2. Prior work suggests that the potent oncogenic potential of vGPCR may, in part, be facilitated by the paracrine secretions of vGPCR-expressing cells (7, 14–16). Indeed, conditioned medium from EC-vGPCR cells showed elevated levels of key Kaposi's sarcoma cytokines, such as interleukin (IL)-6,

murine IL-8 (KC), and stromal cell-derived factor-1 (SDF-1; Fig. 2A). To determine if these paracrine secretions could contribute to the proliferation of latently infected endothelial cells, we treated EC-vCyclin/vFlip cells with supernatants obtained from cultured EC-vGPCR. Surprisingly, conditioned medium from vGPCR-expressing cells promoted the proliferation of endothelial cells expressing KSHV latent genes (Fig. 2B). These results suggested that the paracrine secretions from vGPCR-expressing cells may also promote the tumorigenic potential of latently infected spindle cells.

To study the complex interplay among lytic and latent gene-expressing endothelial cells in Kaposi's sarcomagenesis *in vivo*, we s.c. injected mixed-cell populations of EC-vGPCR along with EC-vCyclin/vFlip into nude mice (Fig. 2C) in a ratio that approximates the proportion of vGPCR-expressing and latent gene-expressing tumor cells found in human Kaposi's sarcoma (3). Coinjection of EC-vCyclin/vFlip (10^6 cells) with a few endothelial cells stably expressing vGPCR (EC-vGPCR; 10^5 cells) synergistically enhanced EC-vCyclin/vFlip tumorigenicity (Fig. 2D). Coexpression of a third latent gene, *LANAI*, failed to further promote tumor growth (data not shown). Immunohistochemical analysis of tumors formed using this allograft model for Kaposi's sarcoma revealed that vGPCR-expressing cells promote the tumoral growth of EC-vCyclin/vFlip, as only few tumor cells expressed vGPCR, whereas the majority of cells expressed these latent genes (Fig. 2E), similar to human Kaposi's sarcoma lesions (9).

vGPCR-expressing endothelial cells can be rendered sensitive to ganciclovir treatment by coexpressing HSV1-TK. These results implicate vGPCR in Kaposi's sarcoma tumor development through the secretion of key Kaposi's sarcoma cytokines and growth factors, raising whether specifically targeting the vGPCR-expressing cells, thereby quenching the secretion of these paracrine growth factors, could be an effective approach for treating established Kaposi's sarcoma lesions. To address this question, we set out to establish a vGPCR-expressing cell line that could be selectively and specifically targeted *in vivo*. To this end, we took advantage of the fact that the HSV1-TK renders expressing cells exquisitely sensitive to treatment with acyclic guanine analogues (e.g., ganciclovir; ref. 17). We employed a bicistronic construct encoding both vGPCR and HSV1-TK (Fig. 3A) to ensure that all cells expressing vGPCR coexpressed HSV1-TK and were therefore rendered sensitive to ganciclovir treatment. We first confirmed coexpression of both genes in transiently transfected cells (data not shown) before generating an endothelial cell line stably expressing this bicistronic construct (EC-vGPCR/TK). We next tested the effects of increasing doses of ganciclovir on the EC-vGPCR/TK cell line. Proliferation of EC-vGPCR/TK, as determined by incorporation of [3 H]thymidine, was similar to that of EC-vGPCR during the same time interval (data not shown), suggesting that coexpression of the HSV1-TK in the absence of ganciclovir did not affect the proliferative potential of EC-vGPCR/TK. Conversely, proliferation of EC-vGPCR/TK in the presence of ganciclovir was dramatically reduced (Fig. 3B), with an IC_{50} of ~1 μ mol/L compared with ~1,000 μ mol/L for EC-vGPCR. Extended treatment of EC-vGPCR/TK with 10 μ mol/L ganciclovir induced cell death in ~100% of treated cells within 4 days (Fig. 3C); terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assays revealed that cell death was not through apoptosis (data not shown). Conversely, EC-vGPCR cells

were completely insensitive to this dose of ganciclovir and continued to proliferate until reaching confluence (Fig. 3C). Collectively, these results suggest that the EC-vGPCR/TK cell line is specifically and exquisitely sensitive to cell death in the presence of ganciclovir.

vGPCR/TK-expressing endothelial cells are sensitive to ganciclovir treatment *in vivo*. We next set out to determine if EC-vGPCR/TK was sensitive to ganciclovir treatment *in vivo*. To this end, we established tumor allografts by injecting EC-vGPCR/TK s.c. into nude mice. Tumors formed from EC-vGPCR/TK grew rapidly and were similar in size to those formed from EC-vGPCR during the same time interval (Fig. 4A), suggesting that coexpression of the HSV1-TK in the absence of ganciclovir did not affect the ability of vGPCR-expressing cells to induce tumors. However, treatment of animals with established EC-vGPCR/TK tumors (100 mg) with a tolerable dose of ganciclovir (50 mg/kg/d; ref. 18) for 4 consecutive days completely abolished tumor growth and induced tumor regression (Fig. 4A), with only residual scar tissue remaining up to 2 months after treatment (data not shown). Conversely, EC-vGPCR formed tumors in the presence or absence of ganciclovir, confirming the specificity of the targeted cells

in vivo. Immunohistochemical analysis of tumors formed from EC-vGPCR/TK and treated for 4 consecutive days with ganciclovir revealed a complete loss of all vGPCR-expressing cells within 1 day of the completion of the treatment cycle (Fig. 4B). Conversely, vGPCR-expressing cells were readily detected in tumors formed from EC-vGPCR and treated with ganciclovir (Fig. 4B). Collectively, these results show that coexpression of HSV1-TK in vGPCR/TK-expressing cells renders these cells sensitive to treatment with ganciclovir *in vivo*.

Selectively targeting only rare vGPCR-expressing tumor cells in mixed-cell tumors composed primarily of cells expressing latent KSHV genes induces tumor regression. We next set out to determine if targeting the few vGPCR-expressing cells in a tumor formed from mixed-cell populations of EC-vGPCR/TK with EC-vCyclin/vFlip could affect the growth of tumors formed primarily from cells expressing the KSHV latent genes, *vCyclin* and *vFlip*. To this end, we coinjected 10^6 EC-vCyclin/vFlip cells with a smaller number (10^5) of endothelial cells stably expressing vGPCR and HSV1-TK (EC-vGPCR/TK; Fig. 5A). Similar to the EC-vGPCR cell line, EC-vGPCR/TK synergistically enhanced EC-vCyclin/vFlip tumorigenicity (Fig. 5B).

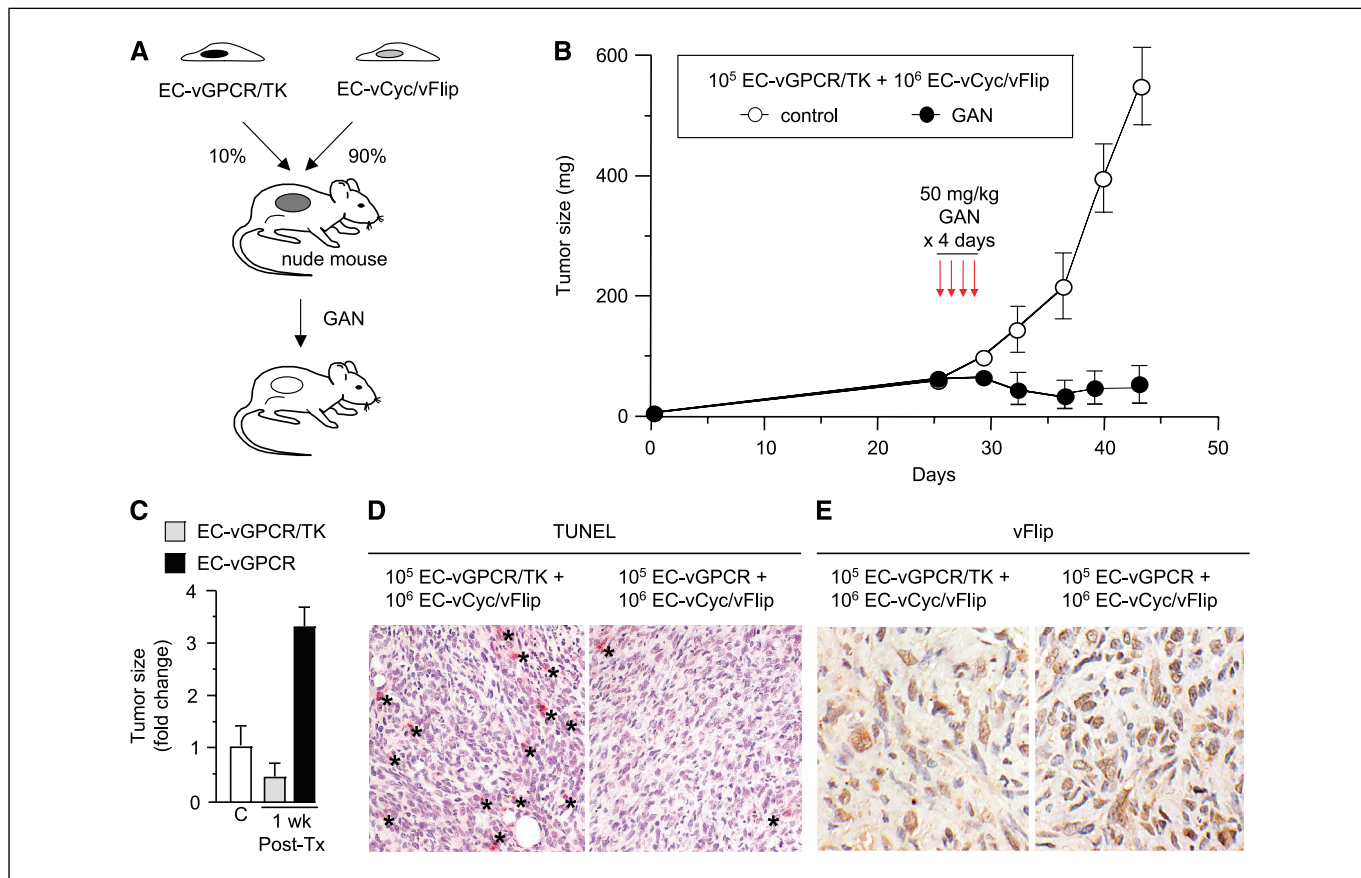


Figure 5. Selectively targeting only rare vGPCR-expressing tumor cells is sufficient to induce tumor regression. *A*, schematic representation of experiment in which tumors formed from the s.c. injection of 10^5 EC-vGPCR/TK cells with 10^6 EC-vCyclin/vFlip cells are treated with ganciclovir to specifically target only the vGPCR-expressing cells. *B*, established tumors arising from mixed-cell populations of 10^5 EC-vGPCR/TK cells with 10^6 EC-vCyclin/vFlip cells treated with PBS (control) or ganciclovir (50 mg/kg) for 4 consecutive days when they reached ~50 mg. Tumor weight was calculated ($LW^2/2$, where L and W represent the longest length and shortest width of the tumor, respectively) and recorded biweekly. *C*, size of tumors arising from mixed-cell populations of 10^5 EC-vGPCR or 10^5 EC-vGPCR/TK cells with 10^6 EC-vCyclin/vFlip 1 week after treatment with ganciclovir (1 wk Post-Tx). Columns, mean fold induction with respect to tumor size before treatment; bars, SD. *D*, representative TUNEL analysis showing an increase in apoptotic cells (*) within tumors generated by the indicated mixed-cell populations 1 week after treatment with ganciclovir. Original magnification, $\times 20$. *E*, immunohistochemical staining showing expression of KSHV latent genes within these tumors 1 week after treatment with ganciclovir. Original magnification, $\times 40$.

Surprisingly, although only few of the tumor cells expressed vGPCR, treatment of animals with established tumors with ganciclovir (50 mg/kg) for 4 consecutive days induced tumor regression (Fig. 5B) and sustained inhibition of tumor growth for up to 4 weeks following treatment (data not shown). In contrast, the small tumors formed from EC-vCyclin/vFlip alone were unaffected by treatment with ganciclovir (data not shown). Furthermore, the administration of ganciclovir did not prevent the rapid growth of tumors arising from EC-vCyclin/vFlip cells mixed with EC-vGPCR, which served as a specificity control (Fig. 5C). Immunohistochemical analysis of tumors 1 week after treatment revealed an increase in apoptosis in the remaining tumor cells in tumors that included EC-vGPCR/TK cells (Fig. 5D). To further investigate the effect of specifically targeting only the rare vGPCR-expressing cells on tumor cells expressing KSHV latent genes, we examined tumors 1 week after treatment of animals with 4 consecutive days of ganciclovir, after which EC-vGPCR/TK cells could no longer be detected (Fig. 3B; data not shown). Surprisingly, immunohistochemical staining indicated that although ganciclovir treatment halted tumor growth a reduced number of cells expressing KSHV latent genes could be detected (Fig. 5E), suggesting that the remaining cells expressing KSHV latent genes are not sufficient for tumor growth in the absence of the paracrine secretions from vGPCR-expressing cells. These results suggest that established Kaposi's sarcoma tumors may be dependent on the presence of the rare vGPCR-expressing cells for tumor growth.

Discussion

Despite over a century since its initial description, Kaposi's sarcoma remains a poorly understood disease. The recent discovery of KSHV as the viral etiologic agent of Kaposi's sarcoma has exposed many potential therapeutic targets. Among all candidate oncogenes encoded by KSHV, only vGPCR has been thus far shown to induce Kaposi's sarcoma-like lesions in several independent transgenic animal models (3–5). Indeed, emerging evidence suggests that dysregulated expression of this potent oncogene in nonlytic cells may represent an early event initiating Kaposi's sarcomagenesis (6). Nonetheless, as expression of vGPCR is associated with only a subpopulation of spindle cells in Kaposi's sarcoma animal models and in human Kaposi's sarcoma, it is possible that the expression of this receptor may create an environment permissive for the subsequent tumor development driven by other KSHV survival (latent) genes, after which receptor expression is no longer necessary. This "hit-and-run" mechanism would imply that although

vGPCR could be an attractive therapeutic target in preventing the initiation of Kaposi's sarcoma it might not be an appropriate target for the treatment of established Kaposi's sarcoma lesions.

Surprisingly, however, using a Kaposi's sarcoma allograft mouse model and a novel approach to specifically eliminate pharmacologically all vGPCR-expressing cells, we obtained evidence here that the paracrine secretions from the few vGPCR-expressing cells in established Kaposi's sarcoma lesions may still be required to promote growth of established Kaposi's sarcoma lesions. Of note, the role of vGPCR-expressing cells in paracrine-driven tumorigenesis is not without precedent. A similar function has been attributed previously to Reed-Sternberg cells in Hodgkin's lymphoma. Thus, tumor cells expressing vGPCR, although rare, might serve as vulnerable targets in established Kaposi's sarcoma lesions. Indeed, as cellular GPCRs are the target of ~60% of all current pharmaceutical drugs, vGPCR and its downstream effectors (11–16, 19, 20) represent attractive candidates for the development of novel therapies for Kaposi's sarcoma.

Of note, despite evidence of an increased rate of apoptosis in mixed-cell tumors in which vGPCR-expressing cells have been pharmacologically removed, few tumor cells still persisted after ganciclovir treatment, which expressed vCyclin and vFlip. These cells may have been protected from apoptosis by the prosurvival effect of these KSHV latent genes. Nonetheless, these remaining cells did not regrow tumors even after prolonged observation (2 months). Collectively, these results, along with prior work, suggest a model in which vGPCR plays a key role in the early events of Kaposi's sarcomagenesis by triggering endothelial cell transformation and promoting the subsequent tumoral growth of cells expressing KSHV latent genes. Ultimately, a collaborative approach to Kaposi's sarcoma treatment in which antiviral and antiangiogenic therapies are combined with gene product-targeted therapies directed against key KSHV latent and lytic genes may ultimately prove to be the most effective therapeutic strategy for the treatment of patients suffering from this still enigmatic and disabling disease.

Acknowledgments

Received 3/25/2005; revised 9/5/2005; accepted 10/18/2005.

Grant support: In part by the Intramural Research Program of the National Institutes of Health, NIDCR.

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