Chemotherapy Induces Lytic EBV Replication and Confers Ganciclovir Susceptibility to EBV-positive Epithelial Cell Tumors

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ABSTRACT

EBV is an oncogenic herpesvirus associated with a number of human malignancies. The consistent presence of the EBV genome in certain tumors offers the potential for novel EBV-targeted therapies. EBV can infect cells in either a latent or lytic form. Here we demonstrate that a variety of chemotherapeutic agents, including cis-platinum, 5-fluorouracil (5-FU), and taxol, induce the switch from the latent to lytic form of EBV infection in tumor cells. This effect requires the protein kinase C δ, phosphatidylinositol 3-kinase, and p38 stress mitogen-activated protein kinase signaling pathways but not caspase 3 activation. Because the lytic but not latent form of EBV infection converts the cytotoxic prodrug, ganciclovir (GCV), into its active form, we examined whether the combination of GCV and chemotherapy is more effective than chemotherapy alone for killing EBV-positive tumor cells. GCV significantly enhanced the ability of 5-FU and cis-platinum to kill EBV-positive, but not EBV-negative, gastric carcinoma cells in vitro. Most importantly, the combination of GCV and 5-FU (or GCV and cis-platinum) was much more effective in the treatment of EBV-positive nasopharyngeal carcinomas passaged in nude mice than either agent alone. These data suggest that GCV enhances the efficacy of conventional chemotherapy for the treatment of EBV-positive epithelial cell tumors.

INTRODUCTION

EBV is a widespread human γ herpesvirus, which causes infectious mononucleosis and is associated with various malignancies, including Hodgkin’s Disease, NPC, B-cell lymphomas, and gastric carcinomas (1, 2). Regardless of whether EBV is an important etiological factor in these malignancies or is merely a passenger virus, the fact that the EBV genome is present in all of the EBV-positive tumor cells, but only a small subset of normal B cells, suggests that development of novel approaches to specifically kill EBV-infected cells is warranted.

As is the case for all herpesviruses, EBV can infect cells in either a latent or lytic form. In cells containing the lytic type of EBV infection, the viral genome is replicated using a virally encoded DNA polymerase (1, 2), and virally encoded kinases are expressed that phosphorylate GCV, thus inducing “bystander” killing (7). Consistent with these properties, the introduction of the HSV-tk, which phosphorylates GCV, into only a portion of tumor cells, combined with systemic GCV treatment, is an effective method for treating certain animal tumor models (6, 7). Because EBV-positive tumor cells are primarily infected with the latent form of EBV infection, GCV by itself is not useful for treating EBV-positive tumors.

However, EBV-positive tumor cells could be targeted potentially for specific destruction by GCV if the latent EBV infection present in the majority of tumor cells could be switched into the lytic form (3, 8–11). The switch from the latent to lytic form of EBV infection can be induced by expression of either EBV IE protein (BZLF1 or BRLF1; Refs. 12–17). Both BZLF1 and BRLF1 are transcription factors, and each IE protein activates transcription of the other (1, 16, 18–21). Together, BZLF1 and BRLF1 are sufficient to induce the entire program of lytic EBV gene expression. In vitro, a variety of different treatments, including phorbol ester, sodium butyrate, transforming growth factor-β, and activation of the B-cell receptor, induce the lytic form of EBV infection (1, 2) by activating BZLF1 and/or BRLF1 transcription. In addition, γ-irradiation and butyrate induce the lytic form of EBV infection in B-cell lymphomas in vivo (8), although the precise mechanism for this effect has not been well clarified.

A number of the agents shown previously to induce the lytic form of EBV infection in vitro are clearly stressful to the host cell and concomitantly induce apoptosis (22). In addition, activation of the lytic form of EBV infection induced by either expression of the BRLF1 IE protein, or by engagement of the B-cell receptor, requires the p38 stress MAPK signaling pathway (19). These findings suggest that the stress-inducing chemotherapeutic agents commonly used to treat EBV-positive malignancies might induce the lytic form of EBV infection in tumor cells.

In this study, we demonstrate that a variety of chemotherapeutic agents with diverse mechanisms (cis-platinum, 5-FU, and taxol) each induce the lytic form of EBV infection in tumor cells and that this effect requires the p38 stress MAPK, PI3 kinase, and protein kinase C δ signaling pathways. Furthermore, we demonstrate that treatment of EBV-positive, but not EBV-negative, tumor cells with chemotherapy in vitro concomitantly converts the cells to a GCV-sensitive phenotype. Most importantly, we show that the combination of GCV and 5-FU, or GCV and cis-platinum, is much more effective in treating EBV-positive NPC tumors in nude mice than either agent alone. Our data suggest that GCV treatment could potentially enhance the therapeutic efficacy of conventional chemotherapy for EBV-positive epithelial cell tumors.

MATERIALS AND METHODS

Cell Lines. Akata is an EBV-positive Burkitt lymphoma cell line (23). AGS is a gastric carcinoma cell line. The AGS-EBV cell line (a gift from Lindsey Hunt-Fletcher) was obtained by G418 selection of AGS cells that were infected with a recombinant Akata virus, in which a neomycin resistance cassette had been inserted into the nonessential BDLF3 open reading frame. Akata cell lines were maintained in RPMI 1640 supplemented with 10% FBS.
AGS cells were maintained in Ham's F-12 medium with 10% FBS, and the AGS-EBV cell line was maintained in Ham's F-12 medium with 10% FBS and 400 μg/ml G418.

**FACS Analysis.** AGS-EBV cells treated with no drug, cis-platinum (1 μg/ml; American Pharmaceutical Partners, Inc.), 5-FU (5 μg/ml; Pharmacia & Upjohn Co.), or taxol (10 nm; Sigma Chemical Co.) were fixed with 40% cold acetone for 10 min on ice and then incubated with a 1:100 dilution of primary antibody (BZLF1; Argene) for 1 h at room temperature, followed by incubation with a 1:100 dilution of FITC-conjugated antimouse IgG (GAM-FITC; Sigma Chemical Co.) for 1 h at room temperature. FACS analysis was performed using a Becton Dickinson FACS machine.

**Immunoblot Analysis.** AGS-EBV cells or Akata cells were treated with either cis-platinum, 5-FU, or taxol at different concentrations for 48 h. Immunoblot analysis was performed subsequently as described previously (19) using anti-BMRF1 (1:100; Capricon), anti-BZLF1 (1:100; Argene), anti-BRLF1 (1:100; Argene), and anti-β-actin (1:5000; Sigma Chemical Co.) antibodies. Results were visualized using a chemiluminescence kit (Amersham).

**In Vivo Chemotherapy Killing Experiments.** AGS and AGS-EBV cells were grown to 70% confluence and treated (in triplicate for each condition) with no drug, cis-platinum (1 μg/ml) or 5-FU (5 μg/ml alone, GCV alone (10 μg/ml), or cis-platinum or 5-FU combined with GCV. The number of viable cells was then determined by trypsin blue exclusion 8 days later.

**Signal Transduction Pathway Inhibition Experiments.** AGS-EBV cells were grown to 70% confluence and pretreated for 1 h with either no agent, PI3 kinase inhibitor LY294002 (15 μm; Calbiochem), p38 MAPK inhibitor SB202190 (20 μm; Calbiochem), protein kinase C δ inhibitor Rottlerin (10 μm; Calbiochem), MAP/ERK kinase 1/2 inhibitor PD98059 (50 μm; Calbiochem), or caspase-3 inhibitor z-DEVD-fmk (50 μm; Calbiochem). Cells were then treated for 48 h with or without 5-FU (5 μg/ml) in the presence or absence of the inhibitors above and harvested for immunoblot analysis for BMRF1 expression.

**Tumor Studies.** The C18 NPC tumor, originally derived from a patient, is an EBV-positive NPC that can be passaged in nude mice (24). Small minced pieces of C18 tumors were transplanted into the flanks of 5–6-week-old female nude mice using Matrigel as described previously (24). To determine whether chemotherapy can induce the lytic form of EBV infection in vivo, mice with C18 NPC tumors were treated with no drug, one dose of cis-platinum (8 mg/kg body weight, i.p.), or 5-FU (75 mg/kg body weight, i.p.). Later (48 h), mice were euthanized, and tumors were removed surgically. Tumor pieces were sonicated in lysis buffer containing 10 mM Tris-HCl (pH 8.0), 1% SDS, 5% β-mercaptoethanol, and 1 × proteinase inhibitors. The sonicated material was boiled for 10 min and centrifuged before being subjected to immunoblot analysis as above.

To determine whether GCV treatment enhances chemotherapy killing of NPC tumors, mice were treated (starting 9 days after transplantation, when most tumors were barely palpable) with one of the following: no treatment (eight tumors), one dose of cis-platinum alone (75 mg/kg; i.p.; Pharmacia & Upjohn Co.; eight tumors), GCV alone (100 mg/kg i.p./day for 5 days; i.p.; Warner-Lambert Co.; eight tumors), or a combination of GCV and one dose of 5-FU (eight tumors). A separate experiment was also performed identical to the one described above, except that cis-platinum (4 mg/kg/i.p.) was substituted for 5-FU. The mice were examined, and tumor measurements obtained three times per week after drug treatment were initiated. Mice were euthanized when tumor size exceeded 1 cm$^3$. After mice were euthanized, both flanks were explored surgically, and any tumors were removed and weighed. Statistical analysis was performed using $t$ test.

**RESULTS**

**5-FU, cis-Platinum, and Taxol Induce Lytic EBV Infection in Vitro.** The ability of 5-FU, cis-platinum, and taxol to induce the lytic form of EBV infection was examined in vitro in both an EBV-positive gastric carcinoma cell line (AGS-EBV) and an EBV-positive Burkitt lymphoma line (Akata). Cells were treated with 5-FU, cis-platinum, or taxol at different concentrations, and 2 days later, immunoblot analysis was performed to quantitate the expression of the EBV IE proteins (BZLF1 and BRLF1) or the early protein BMRF1. The three chemotherapy drugs significantly induced the expression of lytic EBV proteins in both AGS-EBV (Fig. 1, a and b) and, to a lesser extent, Akata cells (Fig. 1c). FACS analysis was also performed to quantify the number of cells converted to the lytic type of EBV infection after chemotherapy treatment of AGS-EBV cells (Fig. 2). Whereas untreated AGS-EBV cells had ~4% of cells expressing the lytic protein, BZLF1, AGS-EBV cells treated for 2 days with cis-platinum, 5-FU, or taxol had 24–28% of cells expressing BZLF1. Thus, a variety of chemotherapeutic agents can induce the lytic form of EBV in a significant portion of cells.

**Involvement of Signal Transduction Pathways in the Induction of Lytic EBV Infection by Chemotherapy Drugs.** To begin to define the mechanism(s) by which chemotherapy drugs induce the lytic form of EBV infection, the effect of various agents known to inhibit specific signal transduction pathways was examined. Both the MAPK/ERK and stress MAPK pathways have been shown previously to play a role in the induction of lytic EBV infection by certain agents (19, 25, 26). Pretreatment of AGS-EBV cells with a specific p38 MAPK inhibitor (SB202190) greatly decreased the ability of 5-FU to induce expression of BMRF1 (Fig. 3a), while having essentially no effect on the level of constitutive BMRF1 expression. A similar effect was observed in cis-platinum-treated cells (data not shown). Pretreatment of AGS-EBV cells with a MAPK/ERK kinase inhibitor (PD98059) also reduced induction of BMRF1 expression by both 5-FU (Fig. 3a) and cis-platinum (data not shown). Thus, the p38 stress MAPK signaling pathway and, to a lesser extent, the MAPK/ERK pathway contribute to the induction of lytic EBV infection by chemotherapy drugs.

Protein kinase C has been shown to be involved in the activation of lytic EBV infection by certain agents (1, 26), and a number of chemotherapy drugs has been shown recently to activate the protein kinase C δ isoform (27, 28). In addition, we demonstrated recently that activation of PI3 kinase is required for induction of lytic EBV infection by certain stimuli (29). Pretreatment of AGS-EBV cells with a specific inhibitor of protein kinase C δ (Rottlerin) almost completely blocked 5-FU-induced BMRF1 expression (Fig. 3b) and had similar effects on cis-platinum-induced BMRF1 (data not shown). Pretreatment of AGS-EBV cells with a PI3 kinase inhibitor (LY294002) also reduced the effect of 5-FU and cis-platinum (Fig. 3b and data not shown). However, treatment of cells with two different caspase inhibitors (the specific caspase 3 inhibitor, Z-DEVD-fmk, and the pan-caspase inhibitor, Z-VAD-fmk) had no effect (Fig. 3b and data not shown). Thus, the protein kinase C δ and PI3 kinase signaling pathways appear to be involved in the activation of EBV by chemotherapeutic drugs. However, apoptosis per se does not appear to be required, as caspase inhibitors had no effect.

**GCV Enhances cis-Platinum and 5-FU Toxicity in an EBV-dependent Manner.** The ability of cis-platinum and 5-FU to induce the lytic form of EBV infection in at least a portion of AGS-EBV cells suggests that these drugs would also enable the cells to phosphorylate GCV into its active cytotoxic form. This suggests that the combination of GCV and chemotherapy might be more effective than either agent alone, as phosphorylated GCV could potentially produce bystander killing in tumor cells where the lytic form of EBV infection was not induced by chemotherapy and in addition could potentially enhance the cell killing effect of the chemotherapy agents. To test this possibility, EBV-negative or -positive AGS cells were treated with either no drug, cis-platinum or 5-FU alone, GCV alone, or the combination of chemotherapy and GCV. As expected, GCV alone was not significantly toxic to AGS cells in the presence or absence of the EBV genome (Fig. 4a), cis-platinum (1 μg/ml) or 5-FU (5 μg/ml) alone, as expected, were cytotoxic in both the EBV-positive and -negative AGS cells (killing 50–75% of cells; data not shown). Importantly, in comparison with the number of cells surviving chemotherapy alone
The number of cells surviving the combination of GCV and cis-platinum, or GCV and 5-FU, was reduced significantly in the EBV-positive AGS cells but not in the EBV-negative AGS cells. Thus, 5-FU or cis-platinum treatment rendered AGS-EBV cells much more sensitive to the cytotoxic effects of GCV, and the enhanced cytotoxicity was EBV dependent.

5-FU and cis-Platinum Induce Lytic EBV Infection in NPCs in Vivo. We next examined whether 5-FU or cis-platinum treatment can induce the lytic form of EBV infection in vivo. C18 NPC tumors were s.c. transplanted into nude mice. When the tumors were readily palpable, animals were inoculated i.p. with either one dose of 5-FU (75 mg/kg body weight), cis-platinum (8 mg/kg body weight), or saline. Two days later, mice were euthanized, and tumor proteins were analyzed by immunoblot analysis. As shown in Fig. 5, both cis-

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Fig. 1. Induction of lytic EBV infection in vitro by chemotherapy agents. EBV-positive gastric carcinoma cells (AGS-EBV, a and b) and Burkitt lymphoma cells (Akata, c) were treated with 5-FU, cis-platinum (CIS), or taxol at different concentrations as indicated. AGS cells are the EBV-negative parental cell line for AGS-EBV cells. Immunoblot analysis was performed after 48 h of drug treatment to quantitate expression of lytic EBV proteins (BZLF1, BRFL1, or BMRF1) versus a cellular protein, β-actin. The anti-immunoglobulin-treated Akata cells (c) serve as a positive control for expression of lytic EBV proteins.

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Fig. 2. Chemotherapy treatment increases the number of cells infected with the lytic form of EBV. AGS-EBV cells were treated for 48 h with or without cis-platinum (1 μg/ml), 5-FU (5 μg/ml), or taxol (10 nm). Cells were then harvested, and the percentage of cells expressing BZLF1 was quantitated by FACS analysis.

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Fig. 3. Multiple signal transduction pathways are required for induction of lytic EBV infection by 5-FU. In a, AGS-EBV cells were pretreated for 1 h with or without a MAPK/ERK kinase inhibitor (PD98059) or p38 MAPK inhibitor (SB202190) in the presence or absence of 5-FU (5 μg/ml). Immunoblot analysis was used to quantitate expression of the lytic EBV protein, BMRF1, 48 h later. b, as in a, except cells were treated with or without a PI3 kinase inhibitor (LY294002), protein kinase C inhibitor (Rottlerin), or a caspase-3 inhibitor (z-DEVD-fmk) before the addition of 5-FU.

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Fig. 4. b and c, the number of cells surviving the combination of GCV and cis-platinum, or GCV and 5-FU, was reduced significantly in the EBV-positive AGS cells but not in the EBV-negative AGS cells. Thus, 5-FU or cis-platinum treatment rendered AGS-EBV cells much more sensitive to the cytotoxic effects of GCV, and the enhanced cytotoxicity was EBV dependent.
platinum and 5-FU induced expression of the EBV IE proteins, BZLF1 and BRLF1, in NPC tumors. Thus, clinically relevant doses of chemotherapy can convert the EBV genome into the lytic form of infection in at least a portion of NPC tumor cells in vivo.

GCV Enhances the Effect of 5-FU and cis-Platinum Treatment of NPC in Nude Mice. The physiological significance of the in vitro killing experiments (Fig. 4) was then assessed in vivo using the C18 NPC tumor model in nude mice. Nine days after transplantation of tumors, at which point the majority of tumors was palpable, animals received either no drug, one dose of 5-FU alone (75 mg/kg body weight, i.p.), 5 days of GCV alone (100 mg/kg body weight i.p. twice a day), or one dose of 5-FU followed by 5 days of GCV treatment (Fig. 6a). In a separate experiment, the effect of a single dose of cis-platinum (4 mg/kg body weight i.p.), with or without subsequent GCV treatment, was likewise compared with no drug or GCV treatment alone (Fig. 6b). Although we observed previously that GCV alone has no effect on the growth of EBV-positive lymphomas (8), GCV alone had a small but reproducible effect on the size of C18 NPC tumors, presumably reflecting the propensity of NPC tumors to constitutively express the lytic form of EBV infection in a small portion of tumor cells (30, 31). NPC tumors in mice treated with 5-FU or cis-platinum alone, as expected, were also smaller than the tumors in the untreated mice. Most importantly, however, the tumors in mice treated with GCV and 5-FU together, or GCV and cis-platinum together, were significantly smaller than the tumors in mice treated with 5-FU (P = 0.02, t test) or cis-platinum (P = 0.003, t test) alone.

We also compared the total number of tumors at the end of the experiment (day 30) in each treatment group. In this animal model system, tumor take is not 100% because some of the tumors spontaneously regress. Nevertheless, the number of tumors discovered at autopsy was similar for mice treated with no drug, GCV alone, 5-FU alone, or cis-platinum alone but was much reduced in the mice treated with either 5-FU and GCV or cis-platinum and GCV (Fig. 6c). These results suggest that GCV significantly enhances the therapeutic efficacy of 5-FU and cis-platinum for treating EBV-positive NPC.

DISCUSSION

The clinical importance of EBV-positive tumors has increased with the onset of the AIDS epidemic and the use of immunosuppressive drugs for organ transplants. Furthermore, EBV infection has been associated recently with a growing number of tumor types. In addition to its very frequent presence in the B-cell lymphomas (32) and leiomyosarcomas (33) of immunosuppressed patients, EBV is virtually always present in undifferentiated NPC (34), commonly present in Hodgkin’s Disease (35), and sometimes present in T-cell lymphomas (36), gastric carcinomas (37), and possibly breast carcinomas (38). The presence of the EBV genome in certain tumors suggests that EBV-based strategies could be used to treat such tumors. Here we demonstrate that a variety of chemotherapy drugs have the unexpected
ability to convert the latent type EBV infection normally present in tumor cells into the lytic form, thereby activating expression of EBV-encoded proteins that phosphorylate the prodrug GCV into its active cytotoxic form. Furthermore, we show that chemotherapy treatment confers GCV susceptibility to tumors in an EBV-dependent manner. Our results suggest that the treatment of EBV-positive tumors in patients could potentially be enhanced by the addition of GCV to conventional chemotherapy drugs.

Overexpression of either EBV IE protein (BZLF1 and BRLF1) in latently infected tumor cells induces the lytic form of EBV infection (3). Although we have not defined here the exact pathway by which chemotherapeutic agents induce lytic EBV infection, it is likely to occur through activation of IE gene transcription. Consistent with this, our results indicate that expression of both the BZLF1 and BRLF1 IE proteins is increased in AGS-EBV cells treated with chemotherapy in vitro, as well as NPC tumors treated with chemotherapy in vivo. We did not identify conditions that differentially regulate the expression of the two IE proteins and, thus, cannot be certain whether chemotherapy primarily activates the BZLF1 versus BRLF1 IE promoter or activates both promoters simultaneously.

However, from our studies using specific inhibitors, it is clear that at least three different signal transduction pathways, including the p38 stress MAPK, PI3 kinase, and protein kinases C δ pathways, are important for the induction of lytic EBV infection by chemotherapy drugs. Phorbol esters, a potent activator of protein kinase C, have long been recognized to induce the lytic form of EBV infection in certain cell lines (1, 2). Recent work from our laboratory has shown that activation of the p38 stress MAPK (19) and PI3 kinase (29) signaling pathways is also required for induction of lytic EBV infection after activation of the B-cell receptor in Akata cells. The cellular transcription factor, ATF-2, activates the BZLF1 promoter through a cAMP-responsive element binding protein-binding motif (19), and phosphorylation of ATF-2 by the p38 stress MAPK has been shown to enhance its transcriptional function (39). Thus, EBV appears to have developed a clever and sensitive system for detecting stress and impending death in the host cell, allowing the virus to convert immediately to the lytic form of infection and subsequently reinfect a healthy cell.

Here we demonstrate that this propensity of the virus to convert to the lytic form of infection in tumor cells stressed by chemotherapy treatment can be used to therapeutic advantage by the addition of GCV. Although it is already well established that the HSV-tk gene introduced into cells by gene delivery techniques renders those cells sensitive to killing by GCV (7), this approach hinges on the ability to specifically and selectively deliver (or express) the viral thymidine kinase gene in tumor cells, a problem that is not readily overcome. In contrast, all EBV-positive tumor cells already contain the EBV genes required for GCV activation, and, thus, delivery of such genes to these tumors is not an issue. Recently, both the EBV thymidine kinase, as well as the EBV homologue of the CMV UL97 protein (BGLF4), were shown to induce GCV phosphorylation when expressed individually in EBV-negative cells (40–42). Therefore, it is not yet entirely clear whether EBV-induced phosphorylation of GCV is primarily mediated by the virally encoded thymidine kinase and/or the BGLF4 gene product, versus the induction of cellular kinases (43), the fact that lytically infected tumor cells phosphorylate GCV reasonably efficiently (3) suggests that this effect can be used therapeutically. A major advantage of activating expression of the lytic EBV proteins which induce GCV phosphorylation is that it results in specific killing of EBV-positive cells.

The major challenge in using GCV to treat EBV-positive tumors will be establishing methods for activating the lytic viral gene program in patients. We reported recently that both γ irradiation, and
sodium butyrate, can induce the lytic form of EBV infection in vivo in certain EBV-positive tumor types (8). The γ irradiation/GCV combination could potentially be useful for treating EBV-positive tumors that are well localized (as is often the case for AIDS-related central nervous system lymphomas). However, the combination of GCV and chemotherapy would likely be more effective than GCV and radiation for treatment of widely disseminated tumors. The potential therapeutic usefulness of the butyrate/GCV combination in patients remains unanswered at this point. The initial results of a Phase I/II study, in which patients with EBV-positive lymphomas resistant to conventional chemotherapy and radiation were given the combination of arginine butyrate and GCV, suggest that this regimen may be promising (44). However, given the likelihood that chemotherapy is more cytotoxic to EBV-positive tumor cells than arginine butyrate, and the fact that these tumors are generally treated with chemotherapy in any event, the combination of chemotherapy and GCV may be more attractive as an initial treatment regimen than the combination of arginine butyrate and GCV.

Here, we have demonstrated that a variety of chemotherapeutic agents, including cis-platinum, 5-FU, and taxol, can induce the lytic form of EBV infection in at least a portion of EBV-positive tumor cells in vitro, as well as in vivo. Although it has been shown previously that the thymidine analogue 5-iododeoxyuridine (45), as well as cis-platinum (46), can induce the lytic form of EBV infection in certain cell lines in vitro, this report is the first to show that disruption of viral latency can be achieved in tumors in vivo using clinically relevant doses of chemotherapeutic agents and to explore the mechanisms for this effect. The induction of lytic EBV infection in only a portion of tumor cells would not necessarily be expected to contribute significantly to the cytotoxic effect of chemotherapeutic agents. However, expression of the HSV-tk gene in only a small portion of tumor cells can result in regression of the entire tumor in the presence of GCV because of the ability of phosphorylated GCV to be transferred to nearby cells (7). Thus, we hypothesized that induction of lytic EBV infection in even a small portion of tumor cells might likewise confer GCV susceptibility to a much larger number of tumor cells. This indeed appears to be the case, as demonstrated by our finding that GCV and 5-FU together, or GCV and cis-platinum together, inhibit NPC tumor growth in vivo much more effectively than either agent alone. The EBV-dependent nature of this effect was confirmed by our in vitro studies showing that only the EBV-positive, and not EBV-negative, gastric carcinoma cells are susceptible to the enhancement of GCV killing by chemotherapy.

The ability of GCV to enhance the efficacy of 5-FU and cis-platinum therapy for EBV-positive tumors may be because of a combination of effects. First and foremost, 5-FU and cis-platinum treatment are required to increase the number of tumor cells able to phosphorylate GCV to its active form. Phosphorylated GCV may be able to then kill tumor cells that are resistant to 5-FU or cis-platinum alone or enhance chemotherapy-mediated killing by inhibiting DNA repair mechanisms. Interestingly, previous studies showed that 5-FU induces synergistic killing with the HSV-tk/GCV combination (47, 48). A similar mechanism may be partly responsible for the synergy between GCV and 5-FU in treating EBV-positive NPC tumors. However, our finding that GCV also enhances cis-platinum treatment of NPC tumors suggests that GCV treatment will likely increase the effectiveness of a wide variety of chemotherapy drugs for treating EBV-positive tumors.

Our results suggest the intriguing possibility that the addition of a relatively nontoxic drug (GCV) could enhance the therapeutic effectiveness of 5-FU and cis-platinum for treating EBV-positive epithelial cell tumors. It remains to be proven whether chemotherapeutic agents are actually effective in inducing the lytic form of EBV infection in patients using clinically relevant doses of these agents. Given that EBV-positive cell lines in vitro are known to have varying susceptibilities to agents that induce lytic EBV infection, it is likely that the ability of particular EBV-positive tumors to be converted to the lytic form of infection will likewise vary depending on the tumor type and chemotherapeutic agent. Ongoing studies will be necessary to determine whether this strategy can be clinically useful in the context of EBV-related malignancies in patients.

ACKNOWLEDGMENTS

We thank Lindsey Hutt-Fletcher of the University of Missouri-Kansas City for the AGS-EBV and AGS cell lines, Natalie Edmund for help with the animal experiments, and Luke Tan at the National University of Singapore for helpful discussions.

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