ABSTRACT

Cancer suicide gene therapy affords the prospect of using the most optimal genes available because the source of the therapeutic gene is often irrelevant. Currently, there are numerous preclinical and clinical trials to develop tumor ablative therapies that use viral, yeast, or bacterial genes. One such gene, the herpes simplex virus type 1 (HSV-1) thymidine kinase (TK) is widely used as a suicide gene in combination with ganciclovir. In the study reported here, a restricted set of random sequences (semi-random) was introduced into the active site of HSV-1 TK, and the resulting variants were selected on the basis of their ability to confer increased ganciclovir or acyclovir sensitivity to Escherichia coli. Sequence analysis demonstrated that functional mutants contained three to five amino acid substitutions that are unique and novel combinations. On the basis of enzyme assay results, three mutants were identified for further analysis in vitro. These three mutants conferred substantial increased sensitivity to both ganciclovir and acyclovir when compared with IC50s of wild-type TK expressing rat C6 glioma cells. One mutant, SR39, was further evaluated in a xenograft tumor model in nude mice. Expression of SR39 in tumors was shown to prevent tumor growth at prodrug dosages that did not affect wild-type HSV-1 TK-expressing tumors. The use of any of these mutants as a suicide gene should provide a more effective and safer alternative to wild-type TK, because lower, less immunosuppressive doses of ganciclovir will be necessary for tumor ablation, and the use of acyclovir may now be possible.

INTRODUCTION

Originally designed as antiviral drugs, ACV3 and GCV have found a new application as prodrugs in suicide gene therapy of cancer. The target for these antiviral drugs, the HSV-1 TK, is currently being used in numerous clinical trials in combination with GCV to ablate tumors in which the gene has been introduced (1–3). This approach functions because of the low substrate specificity that HSV-1 TK displays and the lack of activity the cellular TK demonstrates toward the guanosine nucleoside analogues. HSV-1 TK phosphorylates the analogue, which is then subsequently phosphorylated to the triphosphate by endogenous enzymes (4). Mimicking dGTP, the analogue-triphosphate is incorporated into nascent DNA by DNA polymerase. The molecular properties of the analogue preclude the polymerase from extending incorporated into nascent DNA by DNA polymerase. The molecular properties of the analogue preclude the polymerase from extending.

RESULTS

Mutagenesis. A semi-random mutagenesis approach was used to introduce combinations of amino acid substitutions within the HSV-1 TK active site. The possible codons for each semi-randomized codon are: L159, I or L; I160, A, V, P, or I; A168, D, Y, V or F; and L169, F, Y, L, I, M, N, K, or stop. Because of codon usage, a number of undesired amino acids are also represented in the library, such as a stop codon at position 169. The total possible number of amino acid combinations is 512. To ensure that all mutants were present multiple times, 12,376 clones were plated onto TK selection plates. A total of 1717 colonies were TK positive, indicating that toward ACV, we previously created a large library of multiple amino acid-containing mutants that were screened for altered prodrug activities (5). Extensive characterization and analysis provided us with only a small number of useful mutants. Because only a million mutants from a library containing 64 million variants were screened, only a small subset of all possible substitutions was evaluated. The presumption made is that only a limited portion of the molecular diversity or landscape was analyzed. To tap into the diversity of the earlier library, without having to screen the remaining 63 million clones, we designed a second-generation library to search for new variants with high activities toward GCV and/or ACV. To do this, the sequence of the best few mutants from the initial library were used to design a smaller second-generation library with the anticipation that even better prodrug-activating mutants containing variations of the existing mutants could be identified.

MATERIALS AND METHODS

Library Construction and Selection. Five codons (encoding Leu-159, Ile-160, Phe-161, Ala-168, and Leu-169) were targeted for semi-random sequence mutagenesis. The construction of this library was similar to those described previously (5) with the exception that the oligonucleotides DMO2211 [55-mer; 5'-AGGCGTGAGCCGCTACGTCGCGGCCGCC- GGCCTCAAC(AC)CTTTCGC(GC)(GC)(CT)GACCGCCA-3'] and DMO2212 [50-mer; 5'-ATAACGGTACCGCGCGCGGTACGAC(AC)(ATG)(TA)- (CA)GGCGATGGATGGC-3'] were used to generate the semi-randomized fragment. The parentheses designate the inclusion of equimolar concentrations of the listed nucleotides during oligonucleotide synthesis. The construction of the library and selection for active mutants was essentially as described previously (5, 6). The vector used for construction of the library and for protein overexpression was pET23dmumy (5). The bacterial strain used as a recipient for the randomized library as well as for overexpression of TK protein was BL21(DE3) pLysS, a gift from Dr. W. C. Summers (Yale University, New Haven, CT). Selection of GCV and ACV-sensitive TK mutants was also essentially as described previously (5, 6).

Mammalian Cell Transfections and Prodrug Sensitivity Assays. Stable transfectants expressing wild-type or mutant (SR11, SR26, and SR39) HSV-1 TKs were constructed in rat C6 glioma cells (ATCC CCL-107). The rat C6 glioma cells were maintained and transfected with the vector alone; vector contained wild-type TK and the three mutant HSV-1 TKs as described previously (7).

Pools of transfectants were selected and sorted for green fluorescent protein expression by flow cytometry as described previously (7). Prodrug sensitivity assays were as described (7).
HSV-1 TK (at) residues 159-161 and 168-169

<table>
<thead>
<tr>
<th>Mutants</th>
<th>L</th>
<th>I</th>
<th>F</th>
<th>A</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR4</td>
<td>I</td>
<td>L</td>
<td>Y</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SR11</td>
<td>F</td>
<td>L</td>
<td>F</td>
<td>N</td>
<td>-</td>
</tr>
<tr>
<td>SR15</td>
<td>F</td>
<td>A</td>
<td>Y</td>
<td>Y</td>
<td>-</td>
</tr>
<tr>
<td>SR26</td>
<td>F</td>
<td>A</td>
<td>F</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SR32</td>
<td>F</td>
<td>V</td>
<td>V</td>
<td>M</td>
<td>-</td>
</tr>
<tr>
<td>SR39</td>
<td>F</td>
<td>L</td>
<td>F</td>
<td>M</td>
<td>-</td>
</tr>
<tr>
<td>SR53</td>
<td>F</td>
<td>V</td>
<td>F</td>
<td>Y</td>
<td>-</td>
</tr>
</tbody>
</table>

(·) denotes identity with the wild-type amino acid sequence.

Fig. 1. Deduced amino acid sequence of seven selected semi-random (SR) mutants with increased activity toward GCV and/or ACV. Standard amino acid nomenclature is used.

13.9% of the 512 possible substitutions yielded detectable levels of enzyme activity. Therefore, ~71 amino acid variations from the library of 512 yielded active enzymes. Small colonies (~140) were preferentially picked and restreaked to confirm functional clones. As one might anticipate, mutants that poorly phosphorylate thymidine are unable to grow to the size of wild-type TK-expressing colonies. We had noted earlier that mutants with high $K_m$'s for thymidine were at a distinct kinetic disadvantage to phosphorylate prodrugs presumably because of reduced competition with thymidine for the active site and less feedback inhibition by dTPP, which binds at the thymidine site (7).

To identify the diversity of substitutions that provide functional activity, 64 mutants were sequenced. All mutants were novel combinations and contained three to five amino acid substitutions. Of the 64 sequences, 12 were duplicates and 3 were represented three times. This level of multiple representatives indicates that most, if not all, of the 512 members of this library were screened.

**Identification of Mutants with Increased Prodrug Sensitivity.**

To determine the level of sensitivity to ACV and GCV, the clones were picked into TK selection broth and, after overnight incubation, diluted and spread onto TK selection plates containing different concentrations of prodrug as described elsewhere (5). Approximately 30 mutants conferred increased sensitivity to at least one of the prodrugs. Crude lysates were made from the bacterial cultures and directly assayed for thymidine, GCV, or ACV phosphorylation using a filter binding assay (8). Seven mutants with the desired activities were identified. Fig. 1 shows the deduced amino acid sequence of these seven clones. All seven mutants contain three to five amino acid substitutions that are unique and novel combinations.

**In Vitro Prodrug Sensitivity Assays.**

On the basis of the amount of product generated in GCV and ACV phosphorylation assays in bacterial lysates (data not shown), three mutants (SR11, SR26, and SR39) were selected for prodrug sensitivity assays in mammalian cells. To evaluate the mutants in vitro, mammalian vectors expressing the TK variants (pREP8 D cells. To evaluate the mutants SR39 were selected for prodrug sensitivity assays in mammalian bacterial lysates (data not shown), three mutants (SR11, SR26, and SR39) were identified. Fig. 1 shows the deduced amino acid sequence of a filter binding assay (8). Seven mutants with the desired activities were sequenced. All mutants were novel combinations and contained three to five amino acid substitutions. Of the 64 sequences, 12 were duplicates and 3 were represented three times. This level of multiple representatives indicates that most, if not all, of the 512 members of this library were screened.

**Table 1**

<table>
<thead>
<tr>
<th>Mutants</th>
<th>IC_{50} GCV</th>
<th>Relative to TK</th>
<th>IC_{50} ACV</th>
<th>Relative to TK</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK</td>
<td>3</td>
<td>1</td>
<td>&gt;20</td>
<td>1</td>
</tr>
<tr>
<td>SR11</td>
<td>0.15</td>
<td>33</td>
<td>6</td>
<td>&gt;3</td>
</tr>
<tr>
<td>SR26</td>
<td>0.04</td>
<td>125</td>
<td>0.76</td>
<td>&gt;26</td>
</tr>
<tr>
<td>SR39</td>
<td>0.017</td>
<td>294</td>
<td>0.11</td>
<td>&gt;182</td>
</tr>
</tbody>
</table>

Fig. 2. Prodrug sensitivity assays of TK-expressing rat C6 transfectants to GCV (A) and ACV (B). Stable transfectants containing wild-type thymidine kinase (HSVTK), semi-random mutants (SR11, SR26, or SR39) or vector only were constructed in rat C6 glioma cells and evaluated for prodrug sensitivity as described in “Materials and Methods.” After the addition of Alamar Blue, the plates were scanned in a fluorometer (Labsystems Fluoroskan II) with an excitation wavelength of 544 nm. The fluorescence intensity at 590 nm for each well containing nucleoside analogue was recorded and expressed as a percentage of the value for control wells with no analogue added (n = 8 for each prodrug concentration and controls). Bars, the percentage of SD; zz, death of 50% of the cells.

TK protein concentration in the C6 cell lysates was determined using immunoblots and densitometry. With the wild-type TK protein level set at 1, all of the mutants protein levels were present at higher concentrations that are unique and novel combinations.
levels ranging from 5- to 19-fold higher with SR39 transfecants containing the most TK protein. One of these, SR39, appeared to display a slightly better sensitivity to both ACV and GCV than the other mutants and was therefore chosen for evaluation in a mouse xenograft tumor model.

Xenograft Tumor Model. In the xenograft tumor studies, nude mice were s.c. injected with pools of transfectant cells (5 × 10^3 cells/mouse; n = 5). The cells used for tumor seeding had been transfected with the expression vector (pREPΔ7:dual-GFP), wild-type TK (pREPΔ7:TK-GFP), or SR39 (pREPΔ7:SR39-GFP). After 5 days to allow for tumor formation, the mice were injected i.p. twice a day for 5 days (days 5–9) and for an additional 6 days (days 10–15), tumor diameter was measured every other day (n = 5 for each group).

A comparison of GCV-treated versus saline-treated wild-type TK-expressing tumors indicates no difference between the low GCV dose (GCV 0.5 mg/kg) and saline treatment (P = 0.21; Table 2). However, the high dose of GCV (5 mg/kg) was able to partially limit tumor growth in the wild-type TK-expressing tumors (P = 0.0002). Mean tumor sizes were 7.9, 7.15, and 4.85 mm for mice treated with saline or GCV at 0.5 or 5 mg/kg, respectively. Mice bearing wild-type TK-expressing tumors showed no reduction in tumor size after 5 mg/kg ACV treatment compared with the saline-treated mice (7.8 versus 7.9 mm; P = 0.874). Mice treated with ACV at 25 mg/kg displayed only a slight, albeit significant, difference in mean tumor size of 6.19 mm (P = 0.024), similar to what was seen with vector-transfected tumors at the same ACV dose. There was no difference in mean tumor size in the 25 mg/kg ACV group when wild-type TK-expressing and vector-transfected tumors were compared (P = 0.351).

To compare the effect of the different produgs on tumors expressing either wild-type TK or mutant SR39, the mean tumor size of the prodrug-treated group was divided by the mean tumor size of the respective saline control. The mean tumor size was 6.15 mm for the saline group, 3.65 mm for the ACV at 5 mg/kg group (P = 0.0005), and 2.45 mm for the ACV at 25 mg/kg treatment group (P = 0.0001). There was a significant difference in mean tumor size when mutant SR39-expressing tumors were compared with vector-transfected tumors (P = 0.0001).

Table 3: Ratio of prodrug treated to saline treated mean tumor sizes with P values

<table>
<thead>
<tr>
<th>Prodrug tumor size (mm)</th>
<th>Saline tumor size (mm)</th>
<th>TK vs. SR39 P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCV 0.5 mg/kg</td>
<td>0.905</td>
<td>0.0001</td>
</tr>
<tr>
<td>GCV 5 mg/kg</td>
<td>0.614</td>
<td>0.0001</td>
</tr>
<tr>
<td>ACV 5 mg/kg</td>
<td>0.987</td>
<td>0.0001</td>
</tr>
<tr>
<td>ACV 25 mg/kg</td>
<td>0.784</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

A value of 1 indicates the same mean tumor size in both prodrug and saline treatment groups. P values were determined using the Fisher Protected LSD test.

Table 2: In vivo response of rat C6 tumors expressing wild-type TK or mutant SR39 to saline or produg treatment

<table>
<thead>
<tr>
<th></th>
<th>Mean tumor size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TK</td>
</tr>
<tr>
<td>Saline</td>
<td>7.9 (0.031)*</td>
</tr>
<tr>
<td>GCV 0.5 mg/kg</td>
<td>7.15 (0.21)*</td>
</tr>
<tr>
<td>GCV 5 mg/kg</td>
<td>4.85 (0.0002)</td>
</tr>
<tr>
<td>ACV 5 mg/kg</td>
<td>1.9 (0.0001)</td>
</tr>
<tr>
<td>ACV 25 mg/kg</td>
<td>3.65 (0.0005)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are P values (Fisher Protected LSD test) from comparisons between saline and produg treatment groups.

** This P reflects a comparison between the TK and mutant SR39 tumor sizes treated with saline.
SR39 (P = 0.0001). Clearly, tumors expressing mutant SR39 were much more sensitive to GCV than those expressing wild-type TK. Tumors expressing mutant SR39 displayed a significant response to GCV at both 0.5 and 5 mg/kg, whereas tumors expressing wild-type TK respond to GCV only at 5 mg/kg. This demonstrates that mutant SR39 was significantly more effective at limiting tumor growth with GCV at a dose 10-fold lower than seen with wild-type TK.

Wild-type TK-expressing tumor size was not affected by ACV at 5 mg/kg, whereas mutant SR39-expressing tumors were significantly smaller (P = 0.0001; Table 3). There was also a difference between wild-type TK and mutant SR39-expressing tumor sizes after treatment with the higher ACV level (25 mg/kg; P = 0.0001). These results indicate that mutant SR39 causes a significant growth restriction with ACV whereas wild-type TK-expressing tumors were only marginally restricted at the higher ACV dose.

**DISCUSSION**

The use of HSV-1 thymidine kinase as a suicide gene in combination with ganciclovir has demonstrated promise for tumor ablation in gene therapeutic settings (1–3). To ablate tumors, immunosuppressive doses of GCV are generally required. As a means to not only improve the overall efficacy of the HSV-1 TK/GCV system but to also reduce the requirements of high-dose GCV treatment, we sought to construct novel HSV-1 TKs with abilities to ablate tumors at lower GCV doses and/or to use ACV at clinically relevant levels. Currently, ACV is not effective with HSV-1 TK in gene therapy primarily because of the very high $K_m$ the enzyme displays toward the prodrug ($K_m = 417 \mu M$; Refs. 7 and 9). In this paper, we report the creation of a second-generation, semi-randomized library from which mutants with increased GCV and ACV activities were identified in *E. coli*, evaluated for the ability to confer increased prodrug sensitivity to tumor cells (one of which was further evaluated in a mouse xenograft tumor model) and examined for kinetic changes incurred by the introduction of multiple amino acid substitutions.

We described previously the creation and selection of mutant HSV-1 TKs that conferred enhanced prodrug sensitivity to *E. coli* from a random sequence-derived library of over a million members (5). Because the library was constructed to be randomized 100% at six codons, one would expect the diversity of this library to be on the order of $64 \times 10^6$. Clearly, with a library size of only one million variants, only a small sampling of the molecular diversity of the first library was examined. As a way to tap into the rest of the diversity pool, a semi-random library was constructed based on the amino acid substitutions found in the best TK mutants from the first library. This second-generation library is a scrambling of the substitutions that yielded the desired activities with the hope that combinations with even greater activities would be generated.

Of the mutants that complemented the tk-deficient *E. coli*, seven were identified to have increased activity toward GCV and/or ACV when bacterial lysates were assayed. Sequence analysis of these seven clones is shown in Fig. 1. Previously, we had used molecular modeling to analyze a mutant (mutant 30) created in the first generation library (5) and on which the semi-random library is based (7). Interestingly, mutant 30 contains six amino acid changes and appears to display altered substrate specificity because of the mutation A168Y (alanine to tyrosine at position 168). We suggested that the larger size side chain of the tyrosine causes the neighboring side chains to move and thereby enlarge the active site, presumably leading to greater accessibility of the analogues. Two of the seven mutants identified from the semi-random library contain a tyrosine at position 168. Substitutions at residues 159–161 are less likely to directly impact substrate specificity because those positions lie outside the active site. It is plausible that the phenylalanine substitution found in four of the seven mutants at residue 168 has a similar affect as the tyrosine substitution in mutant 30.

To compare the mutants to the wild-type HSV-1 TK in a tumor cell line, three mutants with improved GCV and/or ACV activity in *E. coli* were subcloned into mammalian expression vectors that were used to transfect rat C6 glioma cells. The results of *in vitro* prodrug sensitivity assays indicate that all three HSV-1 TK mutants substantially increase the sensitivity to both GCV and ACV compared with cells transfected with the wild-type TK (Fig. 2). Because the wild-type TK-expressing cells lack sensitivity to ACV such that an IC$_{50}$ could not be determined, the fold differences in IC$_{50}$ shown in Table 1 are likely an underestimation for all three mutant TK-expressing cells.

From the results of prodrug sensitivity assays, mutant SR39 displayed superior sensitivity to both ACV and GCV and was selected for *in vivo* evaluation. A xenograft tumor model was developed in which pools of transfectant cells were s.c. injected into nude mice. After 5 days to allow for visible tumor formation, the mice were treated by twice-daily injections of saline, GCV, or ACV for 5 days. Growth of individual tumors was monitored every other day during the treatment period and for an additional 9 days (Fig. 3). Treatment with 0.5 mg/kg GCV resulted in no difference in tumor growth of mice bearing wild-type TK-expressing tumors (Table 2). This is in contrast to the group of mutant SR39-bearing tumors that showed a dramatic impediment of tumor growth after treatment with the same GCV dose. Although both wild-type TK- and mutant SR39-expressing tumors responded to GCV at 5 mg/kg, growth of the SR39-expressing tumors was completely prevented, whereas wild-type TK-expressing tumors still grew, albeit somewhat checked. It appears that SR39 prevents tumor growth when GCV is administered at one-tenth the dose needed to slow wild-type TK-expressing tumor growth.

At the low ACV dose (5 mg/kg) used, only mutant SR39-expressing tumor growth was restricted (Fig. 3 and Table 2). Wild-type TK-expressing tumors were marginally restricted by ACV at 25 mg/kg, whereas the growth of mutant SR39-expressing tumors was substantially impeded. Although earlier *in vivo* studies with another HSV-1 TK mutant (mutant 30) demonstrated similar results (5), mutant SR39-expressing tumors were much more responsive to ACV at both prodrug concentrations.

Despite our attempts to isolate pools of stable transfectants with similar HSV-1 TK expression levels by cell sorting, the amount of protein expression for all of the mutants was higher than that of wild-type HSV-1 TK-expressing transfectants. Relative to the wild-type TK-expressing transfectants, the mutant TK transfectant protein levels ranged from 5- to 19-fold higher with SR39 transfectants containing the most TK protein. Chen *et al.* (10) compared the relationship between HSV-1 TK expression level and GCV cytotoxicity in 9L glioma tumor cells and found that clones with different enzyme levels displayed similar sensitivity to GCV. Three different 9L stable transfectants were shown to have 1-, 4.7- and 16.9-fold differences in protein expression levels. They note that the IC$_{50}$s for GCV for these clones are similar, regardless of the level of HSV-1 TK expression. Several investigators (10–13) suggest that there is a threshold level of GCV beyond which the rate of cell killing does not change. Shewach *et al.* (14) have reported enzyme level GCV sensitivity differences but at the 5000-fold range of enzyme level differences (14). Because the differences in protein expression level described here are in the range reported by Chen *et al.* (10), the protein level differences we observed are likely not responsible for the improved prodrug sensitivity of the mutant TKs.

In cell culture experiments, mutants SR11 and SR39 appear to grow at a rate 20–40% less than the wild type, but this difference is minor in comparison to the 33- and 294-fold enhanced susceptibility to...
THYMIDINE KINASE MUTANTS

The activity (5, 7). Kinetic evaluations have been done on these mutants as HSV-1 TK mutants (mutants 30 and 75) with enhanced prodrug sensitivity conferred by the mutants is a result of the characteristics of the mutants themselves rather than protein level or cell growth rate differences.

Previously, we reported the creation and characterization of two HSV-1 TK mutants (mutants 30 and 75) with enhanced prodrug activity (5, 7). Kinetic evaluations have been done on these mutants as well as the three mutants described in this report (7). Because endogenous thymidine within the cell competes with the prodrug for the active site, it is important to consider the ratio of specificity constants for prodrug and thymidine. As a means to compare the earlier mutants (mutants 30 and 75) with those of the semi-random mutants reported here, we used the equation \( \frac{k_{cat}/K_m(\text{prodrug})}{k_{cat}/K_m(\text{thymidine}) + k_{cat}/K_m(\text{prodrug})} \), which takes the thymidine parameter into account. With GCV as the prodrug, mutants 30, 75, SR11, SR26, and SR39 displayed values 66-, 11-, 59-, 53-, and 83-fold higher, respectively, than the wild-type enzyme. With ACV as the prodrug, mutants 30, 75, SR11, SR26, and SR39 displayed 333-, 70-, 373-, 567-, and 233-fold higher values, respectively, compared with the wild-type enzyme. Clearly, when competition for the active site is considered, all five mutant enzymes reveal an enormous kinetic advantage for both prodrugs over the wild-type enzyme. From this vantage, SR39 is the best enzyme with GCV. With ACV, SR26 is the best enzyme, followed by SR11. The kinetic parameters displayed by the mutants reveal a biochemical basis for the enhanced prodrug sensitivity described here and elsewhere (5, 7). From this comparison, mutant SR39 would appear to ablate tumors more effectively in the presence of GCV, whereas mutants SR11 and SR26 might result in greater tumor sensitivity to ACV. Indeed, significant improvements at the kinetic level with the three new mutants compared with mutants 30 and 75 have been achieved.

Further evidence that mutant SR39 has the ability to increase tumor sensitivity to prodrugs is reported in Gambhir et al. (15). Rat C6 glioma cells stably transfected with mutant SR39 demonstrated better accumulation of penciclovir, an analogue closely related to GCV, in cell culture and in tumors than do cells expressing wild-type TK. Furthermore, when the wild-type TK or mutant SR39 is delivered to tumors using an adenovirus delivery system, mutant SR39 more effectively traps the analogue than the wild-type enzyme does. Analysis of mutant SR39 as an alternate to HSV-1 TK in positron emission tomography, a noninvasive imaging technique, demonstrated that mutant SR39 in the presence of \(^{18}\text{F}\)-labeled penciclovir significantly enhanced sensitivity for imaging reporter gene expression in vivo. Taken together, the multiple amino acid substitutions of the semi-random mutants described here result in enormous alterations in substrate specificities that, when expressed in vitro, display major differences in prodrug-mediated cell killing in transfected cell lines. In vivo, mutant SR39 demonstrates superior tumor ablation in the presence of GCV and ACV. The mutants described here (SR11, SR26, and SR39) should provide a significant advantage over the wild-type TK for suicide gene therapy applications in at least three important ways: (a) by enhancing prodrug-mediated cell killing; (b) by reducing the amount of immunosuppressive GCV required for effective cell killing; and (c) by bringing HSV-1 TK-mediated activation of ACV to a clinically relevant level. Furthermore, such mutants could replace the use of wild-type TK in gene therapy other indications including restenosis, graft versus host disease, and HIV infections (16–18) as well as for noninvasive quantitative imaging of tumor cells with positron emission tomography (15).

REFERENCES


* Black and Kokoris, unpublished observation.
Herpes Simplex Virus-1 Thymidine Kinase Mutants Created by Semi-Random Sequence Mutagenesis Improve Prodrug-mediated Tumor Cell Killing

Margaret E. Black, Mark S. Kokoris and Peter Sabo


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