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# Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: An innovative approach for cancer therapy

**(liver cancer/6-methoxypurine arabinonucleoside/varicella-zoster virus/thymidine kinase)**

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**ABSTRACT** **An approach involving retroviral-mediated gene therapy for the treatment of neoplastic disease is described. This therapeutic approach is called "virus-directed enzyme/prodrug therapy" (VDEPT). The VDEPT approach exploits the transcriptional differences between normal and neoplastic cells to achieve selective killing of neoplastic cells. We now describe development of the VDEPT approach for the treatment of hepatocellular carcinoma. Replication-defective, amphotrophic retroviruses were constructed containing a chimeric varicella-zoster virus thymidine kinase (VZV TK) gene that is transcriptionally regulated by either the hepatoma-associated α-fetoprotein or liver-associated albumin transcriptional regulatory sequences. Subsequent to retroviral infection, expression of VZV TK was limited to either α-fetoprotein- or albumin-positive cells, respectively. VZV TK metabolically activated the nontoxic prodrug 6-methoxypurine arabinonucleoside (araM), ultimately leading to the formation of the cytotoxic anabolite adenine arabinonucleoside triphosphate (araATP). Cells that selectively expressed VZV TK became selectively sensitive to araM due to the VZV TK-dependent anabolism of araM to araATP. Hence, these retroviral-delivered chimeric genes generated tissue-specific expression of VZV TK, tissue-specific anabolism of araM to araATP, and tissue-specific cytotoxicity due to araM exposure. By utilizing such retroviral vectors, araM was anabolized to araATP in hepatoma cells, producing a selective cytotoxic effect.**

Somatic cell gene therapy is a rapidly developing therapeutic approach for the treatment of human disease. The first clinical trials involving retroviral-mediated gene transfer and gene therapy are ongoing. These first trials are assessing the ability to correct adenosine deaminase deficiency in severe combined immunodeficiency disease (1) and to modulate tumor-infiltrating lymphocytes in cancer patients (2, 3).

We now describe an approach for the treatment of neoplastic disease involving retroviral-mediated gene therapy. We call this approach "virus-directed enzyme/prodrug therapy" (VDEPT). The VDEPT concept exploits the transcriptional differences between normal and neoplastic cells to selectively kill the cancer cells. An artificial chimeric gene is created that is composed of tissue-specific transcriptional regulatory sequences (TRSs; for review, see ref. 4) linked to the protein coding domain of a nonmammalian enzyme. The nonmammalian enzyme metabolically activates a nontoxic prodrug to a cytotoxic anabolite. If the TRSs are from a tumor-associated gene, such as the hepatoma-associated α-fetoprotein (AFP) gene, then the artificial gene will produce tumor-specific expression of the nonmammalian enzyme and, consequently, tumor-specific production of the cytotoxic anabolite. Our initial choice of activating enzyme developed from the recent observation that 6-methoxypurine arabinonucleoside [9-(β-D-arabinofuranosyl)-6-methoxy-9*H*-purine (araM); Fig. 1] is selectively toxic to varicella-zoster virus (VZV)-infected cells (5). This selectivity results from the fact that araM is a good substrate for VZV thymidine kinase (TK) but a poor substrate for any of the three major mammalian nucleoside kinases. Once monophosphorylated in a VZV-infected cell, araM monophosphate can be further anabolized by cellular enzymes to produce the cytotoxic anabolite, araATP (21) (Fig. 1). If VZV TK can be selectively expressed in tumor cells, then the cytotoxic anabolite, araATP, will be selectively formed in those tumor cells upon treatment with the prodrug araM. We now describe the development of this approach for the treatment of hepatocellular carcinoma (HCC), but it should be appreciated that this approach may be applicable to other tumor types as well.

Artificial, chimeric genes that generate either liver-specific or hepatoma-specific expression of VZV TK were created and placed into a replication-defective, amphotrophic retroviral shuttle vector for tissue delivery. Subsequent to viral infection, these chimeric genes generated tissue-specific expression of VZV TK, tissue-specific anabolism of araM to araATP, and tissue-specific cytotoxicity due to araM exposure.

## MATERIALS AND METHODS

**Materials.** araM, [8-$^{14}$C]araM (49 Ci/mol; 1 Ci = 37 GBq), and α,α,α-trifluorothymidine (TFT) were synthesized at the Wellcome Research Laboratories (Research Triangle Park, NC) (5 and 6). Tritiation of araM ([G-$^{3}$H]araM; 16 Ci/mmol) was performed by Moravek Biochemicals (Brea, CA). The VZV TK gene (7), designated here as 22TK, was provided by J. Ostrove [National Institutes of Health (NIH), Bethesda, MD]. Plasmid 2335A-1 containing essential albumin (ALB) TRSs, which is equivalent to the NB construct (8), was obtained from R. Palmiter (University of Washington, Seattle, WA). Plasmid XM5 containing the N2 vector (9) was obtained from S. Karlsson (NIH). Human AFP TRSs were isolated from plasmid pAF5.1-CAT (10) provided by T. Tamaoki (University of Calgary, Calgary, Canada).

**Cell Lines.** PA317 (CRL 9078), Hep G2 (HB 8065), Hep 3B (Hep 3B2.1-7; HB 8064), H-4-II-E (CRL 1548), WiDr (CCL 218), SW480 (CCL 228), Detroit 551 (CCL 110), and MCF-7 (HTB 22) cells were obtained from the American Type Culture Collection. HuH7 cells were supplied by B. Mason (Fox Chase Cancer Center, Philadelphia); ψ-2 and NIH 3T3

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Abbreviations: VDEPT, virus-directed enzyme/prodrug therapy; TRS, transcriptional regulatory sequence; AFP, α-fetoprotein; ALB, albumin; araM, 6-methoxypurine arabinonucleoside [9-(β-D-arabinofuranosyl)-6-methoxy-9*H*-purine]; araATP, adenine arabinonucleoside triphosphate; VZV, varicella-zoster virus; TK, thymidine kinase; HCC, hepatocellular carcinoma; TFT, α,α,α-trifluorothymidine; HAT, hypoxanthine/aminopterin/thymidine; LTR, long terminal repeat; PCA, perchloric acid.

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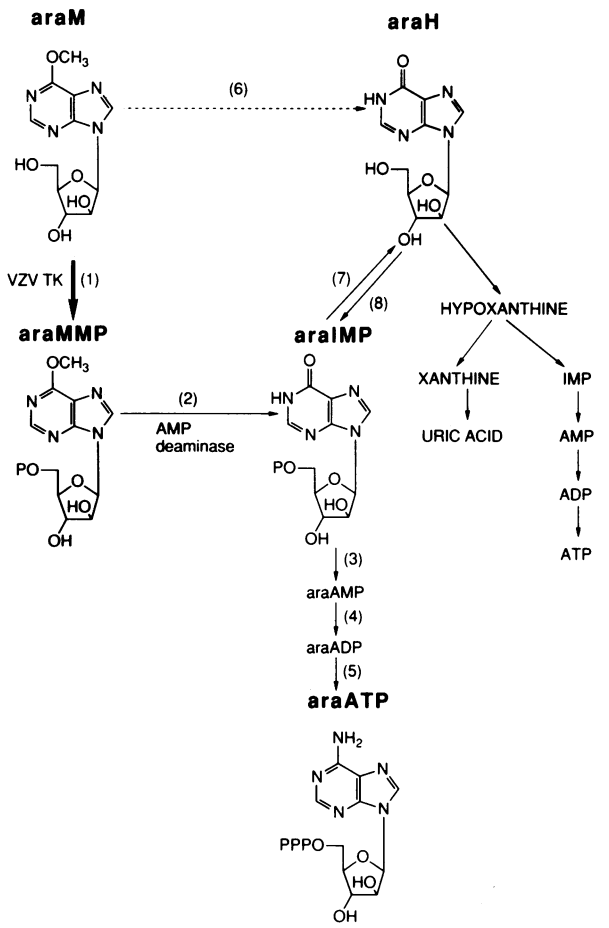


FIG. 1. Metabolism of araM (21). AraM is anabolized to araM monophosphate (araMMP) selectively by VZV TK (step 1). Cellular AMP deaminase (step 2), adenylosuccinate synthetase lyase (step 3), AMP kinase (step 4), and nucleoside diphosphate kinase (step 5) will further anabolize araM monophosphate to adenine arabinonucleoside triphosphate (araATP). Just detectable levels of araATP are formed in the absence of functional VZV TK, presumably because of the presence of adenosine deaminase (step 6) and cellular kinases (step 8). Cellular phosphatases (step 7) may convert araIMP to araH.

$TK^{-/-}$ cells were supplied by S. Karlsson (NIH). For HAT selection, cells were grown in the presence of 100 $\mu$M sodium hypoxanthine, 0.4 $\mu$M aminopterin, and 16 $\mu$M thymidine. For TFT selection, cells were grown in the presence of 10 $\mu$g of TFT per ml of culture medium.

**Construction of VDEPT Retroviral Shuttle Vectors.** A 1376-base-pair (bp) *Acc* I/*Nde* I fragment of the VZV TK gene was isolated from plasmid 22TK. Plasmid 2335A-1 was linearized with *Bam*HI, and the ALB TRS/VZV TK chimera was constructed by inserting the blunt-ended *Acc* I/*Nde* I VZV TK fragment into the blunt-ended *Bam*HI site of 2335A-1, creating plasmid pCR73. The ALB TRS/VZV TK chimera was purified from pCR73 as a 3.8-kilobase (kb) *Sst* I/*Kpn* I fragment. Plasmid pCR74, containing the retroviral shuttle vector CR74 (see Fig. 2), was constructed by inserting the blunt-ended *Sst* I/*Kpn* I fragment from pCR73 into the blunt-ended *Xho* I site of XM5.

To create the AFP TRS/VZV TK chimera, a 3.3-kb *Bam*HI/*Xmn* I fragment was isolated from pCR73 and a 5.5-kb *Xmn* I/partial *Hin*dIII fragment was isolated from pAF5.1-CAT. These two fragments were blunt-ended and ligated to create plasmid pCR77. Plasmid pCR78, containing the retroviral shuttle vector CR78 (see Fig. 2), was constructed by inserting a blunt-ended 6.7-kb *Aat* II/*Pst* I fragment from pCR77 into the blunt-ended *Xho* I site of XM5.

DNA sequencing was performed on double-stranded plasmid DNA by the method of Bartlett *et al.* (11).

**Production of Amphotrophic Recombinant Retroviruses.** Production of replication-defective, amphotrophic retroviral shuttle vectors was accomplished using PA317 cells (12) and $\psi$-2 cells (13) by previously published methods (14). G418-resistant PA317 cells were single cell cloned, and individual clones containing only full-length vector were identified by Southern blot analysis. The following DNA fragments were used as probes: VZV TK probe, a 1.4-kb *Nde* I/*Acc* I fragment from 22TK; AFP probe, a 4.1-kb *Xba* I/*Aat* II fragment from pCR77. Viral titers and infections of target cell lines were performed as described (14), except that 16 $\mu$g of Polybrene per ml was used.

**PCR Analysis.** The ALB TRS/VZV TK and AFP TRS/VZV TK chimeras were amplified using oligonucleotide primers CR8/CR9 and primers CR8/CR10, respectively. CR8 anneals to the 3' end of each chimera. CR9 and CR10 anneal to the promoter regions of the ALB and AFP TRSs, respectively.

**Immunologic and Enzymatic Assays.** Human AFP and ALB production were quantitated using an immunoenzymatic assay (Tandem-eAFP, Hybritech) and ELISA (Albuwell-II, Exocell, Philadelphia, PA), respectively. VZV TK activity was quantitated by [$^{14}$C]araM phosphorylation by a DEAE filter assay as described (5).

**Metabolism of araM.** Cells were plated at a density of $1 \times 10^7$ cells per 150 cm$^2$ on day 0. On day 1, the medium was changed and [$^3$H]araM was added to a final concentration of 10 $\mu$M and a specific activity of 544 mCi/mM. After 48 hr, the cells were washed twice in phosphate-buffered saline (PBS), and 0.4 M perchloric acid (PCA) was added (5 ml per flask) and incubated at 4°C for 30 min. At the time of PCA addition, inosine triphosphate was added (49 nmol per flask) as an internal standard to determine efficiency of extraction procedures. PCA extracts were centrifuged at 800 $\times$ *g* for 5 min at 4°C and the resultant supernatants were neutralized with KOH. The neutralized supernatants were centrifuged at 800 $\times$ *g* for 5 min at 4°C and the resultant supernatants were evaporated to dryness. The residual material was resuspended in 300 $\mu$l of water, and radioactivity was determined by liquid scintillation counting followed by HPLC analysis (21).

**Growth Inhibition Studies.** Inhibition of cell growth was determined using Hoechst 33342 (Sigma) for quantitation of cellular DNA content (15). Cells were plated on day 0 at a density of 4000 cells per well in a 96-well microtiter plate. On day 1, the medium was changed and the indicated compounds were added in 2-fold serial dilutions. On day 3 and day 5, the medium was changed and fresh compounds were added. On day 6, the cells were washed once in serum-free medium and Hoechst 33342 (10 $\mu$g/ml in PBS) was added. After 40 min, the fluorescence was determined using 544 nm and 584 nm as excitation and emission wavelengths, respectively. Concentrations yielding 50% inhibition ($IC_{50}$) were calculated using curve-fitting parameters based on the Marquardt method (16).

## RESULTS AND DISCUSSION

**Recombinant Retroviruses CR74 and CR78.** Plasmids pCR74 and pCR78 contain the recombinant retroviral shuttle vectors CR74 and CR78, respectively (Fig. 2). Both vectors contain two significant TRSs: the 5' viral long terminal repeat (LTR) and either the ALB TRSs (CR74) or AFP TRSs (CR78). The 5' LTR constitutively regulates the expression of the *neo* gene. The neo transcript, as well as the genomic RNA transcript, utilize the poly(A)$^+$ signal located in the 3' LTR. The ALB or AFP TRSs conditionally regulate the expression of the chimeric VZV TK genes utilizing the poly(A)$^+$ site cloned with the coding domain of the VZV TK gene. The *neo* gene and VZV TK genes are in opposite transcriptional

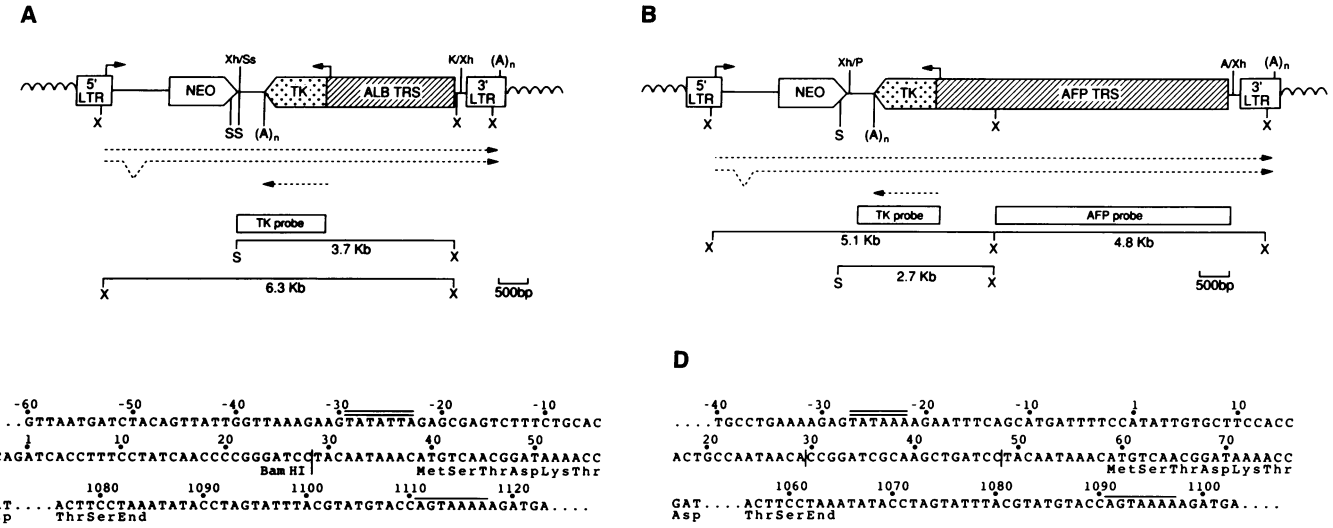


FIG. 2. Structure of VDEPT viruses CR74 and CR78. (*A* and *B*) Diagrams of pCR74 and pCR78, respectively. The VZV TK coding sequence is shown stippled; the ALB TRSs and AFP TRSs are shown hatched. Putative transcripts are shown as dashed lines. Hybridization probes and expected fragment sizes from Southern analysis are shown. ↱, Transcription initiation sites; $(A)_n$, poly(A) sites. A, *Aat* II; K, *Kpn* I; P, *Pst* I; S, *Sal* I; Ss, *Sst* I; X, *Xba* I; Xh, *Xho* I. (*C* and *D*) Partial DNA sequences of pCR74 and pCR78, respectively. Sequences are numbered with the start of RNA transcription as 1. The VZV TK coding sequence is translated [note: The entire VZV TK sequence is not shown since it has been published (17)]. The TATA box is double overlined, the poly(A)$^+$ signal is overlined. (*C*) Bases −60 to 28 are from ALB TRSs; bases 29–1123 are from VZV TK. (*D*) Bases −40 to 29 are from AFP TRSs; bases 30–48 are linker sequences; bases 49–1103 are from VZV TK.

orientation to eliminate non-tissue-specific expression of VZV TK from the 5′ LTR. Based on data from previous reports (18, 19), the putative transcripts generated from the recombinant retroviruses CR74 and CR78 are indicated in

Table 1. Hepatoma and nonhepatoma target cells

| Cell line | Origin | ALB* | AFP* | Genetic alteration† | G418 resistant | VZV TK DNA | VZV TK activity‡ | Cytotoxicity§ araA | Cytotoxicity§ araM |
|---|---|---|---|---|---|---|---|---|---|
| HepG2 | Human liver | 4100 | 2044 | Parental | − | − | 9 | <1 | >2000 |
| | | | | N2 | + | | 4 | <1 | >2000 |
| | | | | CR74 | + | + | 4538 | | 6.5 |
| | | | | CR74¶ | + | | 6600 | | <0.5 |
| | | | | CR78 | + | + | 198 | | 78 |
| | | | | CR78¶ | + | | 1200 | | 3.1 |
| Hep 3B | Human liver | 402 | 311 | Parental | − | − | 2 | 6 | >2000 |
| | | | | CR74 | + | + | 44 | | 367 |
| | | | | CR78 | + | + | 155 | | 135 |
| HuH7 | Human liver | 586 | 1842 | Parental | − | − | 13 | 3 | 1621 |
| | | | | CR74 | + | + | 2831 | | 11 |
| | | | | CR78 | + | + | 200 | | 36 |
| H-4-II-E | Rat liver | ND (+++)‖ | ND (+)‖ | Parental | − | − | 4 | 6 | 1848 |
| | | | | CR74 | + | + | 398 | | 114 |
| | | | | CR78 | + | + | 26 | | 160 |
| MCF-7 | Human breast | BT | BT | Parental | − | − | <1 | <1 | >2000 |
| | | | | CR74 | + | + | <1 | | >2000 |
| | | | | CR78 | + | + | 14 | | 1680 |
| WiDr | Human colon | BT | BT | Parental | − | − | 5 | 15.3 | 1028 |
| | | | | CR74 | + | + | 17 | | 1751 |
| | | | | CR78 | + | + | 7 | | 1744 |
| SW480 | Human colon | BT | BT | Parental | − | − | <1 | <1 | >2000 |
| | | | | CR74 | + | + | <1 | | >2000 |
| | | | | CR78 | + | + | 14 | | 1730 |
| Detroit 551 | Human skin | BT | BT | Parental | − | − | <1 | <1 | >2000 |
| | | | | CR74 | + | + | <1 | | >2000 |
| | | | | CR78 | + | + | 18 | | 1790 |

ND, not determined; BT, below threshold.

*Expressed as ng secreted per mg of cell protein per 4 days.

†Indicated for each cell line, parental unmodified cells (parental) or cells infected with the control virus (N2) (see ref. 13) or with the VDEPT viruses CR74 and CR78.

‡Expressed as pmol of araM phosphorylated per mg of protein per 30 min.

§Micromolar concentration producing a 50% decrease in DNA content after a 5-day exposure. araA cytotoxicity was determined in the presence of 5 μM *erythro*-9-(2-hydroxy-3-nonyl)adenine.

¶A particular clone of either Hep G2/CR74 or Hep G2/CR78 cell lines.

‖Major (+++) and minor (+) amounts of ALB or AFP synthesis based on historical data (20).

Fig. 2. DNA sequence analysis of pCR74 and pCR78 confirmed that the TATA boxes, the ALB or AFP TRS/VZV TK junction, and the entire VZV TK coding domain with its poly(A)$^+$ site were correct (Fig. 2 *C* and *D*).

**Generation of Recombinant Retroviruses.** Genomic DNA derived from clonal lines of PA317/pCR74 or PA317/pCR78 cells were isolated and analyzed by Southern blots to identify clonal lines containing a stably integrated, full-length retroviral vector. Two of the 15 PA317/pCR74 clonal cell lines analyzed contained only a full-length vector. Five of the 25 PA317/pCR78 clonal cell lines analyzed contained only a full-length vector.

**Infection of Target Cells.** Four hepatoma and four nonhepatoma cell lines were used as positive and negative target cells, respectively. AFP and ALB synthesis was measured for each human cell line (Table 1). Hep G2 cells have relatively high synthesis of AFP and ALB and Hep 3B cells have relatively low synthesis of both proteins, whereas HuH7 cells have relatively high AFP synthesis and moderate ALB synthesis. The rat hepatoma cell line H-4-II-E previously was shown to transcribe high levels of ALB (20). None of the human nonhepatoma cell lines had detectable AFP or ALB synthesis.

The full-length VDEPT viruses CR74 and CR78 were used to infect the hepatoma and nonhepatoma target cell lines to assess the tissue-specific expression of VZV TK. Subsequent to infection, cells were selected on G418 (Table 1). Genomic DNA was isolated and analyzed by PCR and DNA slot blots to confirm that all G418-resistant cell lines contained a stably integrated provirus containing a chimeric VZV TK gene (Table 1 and Fig. 3).

VZV TK enzymatic activity was determined in parental and CR74- and CR78-infected target cells. There was a barely detectable apparent rate of araM phosphorylation in cell extracts from control cells (parental) or cells infected with the control N2 virus. This low phosphorylation may result from the sequential action of adenosine deaminase and cellular kinases (Fig. 1) and was considered the background rate in this assay. Activity was increased in CR74- and CR78-infected hepatoma cells compared with the parental hepatoma cells (Table 1). VZV TK expression utilizing either the ALB or AFP TRSs roughly correlated to ALB or AFP production. VZV TK was not significantly increased in CR74- and CR78-infected nonhepatoma cells compared with the parental nonhepatoma cells (Table 1). These data indicate that the VDEPT viruses CR74 and CR78 were able to direct the tissue-specific expression of VZV TK, with expression being roughly correlated to ALB and AFP production.

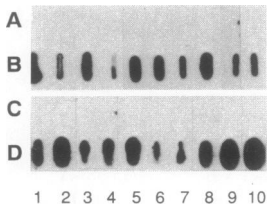


FIG. 3. Slot blot analysis of parental and infected cell lines. Rows A and B, amplified with 1 $\mu$M CR8 (CTCCTGCAGACTGGTACATACGTAAATACTAGG) and 1 $\mu$M CR9 (CTCGAGCTCGGTAAGTATGGTTAATGATCTACAG) specific for ALB TRS/VZV TK; rows C and D, amplified with 1 $\mu$M CR8 and 1 $\mu$M CR10 (CTCCGGTACCCCATTTTCAACCTAAGGAAATACC) specific for AFP TRS/VZV TK. A1–A9 and C1–C9, DNA from parental cells; B1–B9 and D1–D9, DNA from cells infected with CR74 and CR78, respectively. Slots: 1–9, H-4-II-E, HuH7, Hep 3B, Hep G2, WiDR, SW480, MCF-7, Detroit 551, and IMR90, respectively. B10 and D10, pCR74 and pCR78 plasmid DNA, respectively. A10 and C10, no DNA control.

**Metabolism of araM.** To confirm that Hep G2 cells expressing the VZV TK gene can anabolize araM to araATP, parental Hep G2 cells and Hep G2 cells infected with CR74 and CR78 were grown in the presence of [$^3$H]araM. After 48 hr, metabolites of araM were identified in cellular extracts by anion-exchange HPLC analysis. Fig. 4*A* illustrates that there were significant levels of araAMP, araADP, and araATP produced in CR74- and CR78-infected Hep G2 cells compared with Hep G2 parental cells. In addition, ADP and ATP were also labeled to higher specific activity, putatively through the action of cellular phosphatases on araIMP (Fig. 4; see Fig. 1). The levels of araAMP, araADP, and araATP were quantitated and are illustrated in Fig. 4*B*. There were just detectable levels of araATP produced in parental Hep G2 cells after 48 hr of incubation with araM. These low levels presumably were the result of adenosine deaminase activity, which may slowly convert araM to araH (see Fig. 1). These low levels of araATP did not produce any significant cytotoxicity in parental Hep G2 cells (see below). Compared with Hep G2 parental cells, there was an ≈7000-fold and ≈2000-fold increase in araATP levels in Hep G2 cells infected with CR74 and CR78 viruses, respectively.

**AraM-Mediated Growth Inhibition.** The data above suggest that the infected hepatoma cells that express VZV TK may now become selectively sensitive to agents that require VZV TK for efficient anabolism to cytotoxic metabolites. All cell lines experienced approximately equal growth-inhibitory effects resulting from araA exposure, indicating that if araATP was produced, these cells would exhibit a cytotoxic effect (Table 1). However, only the hepatoma cells that had signif-

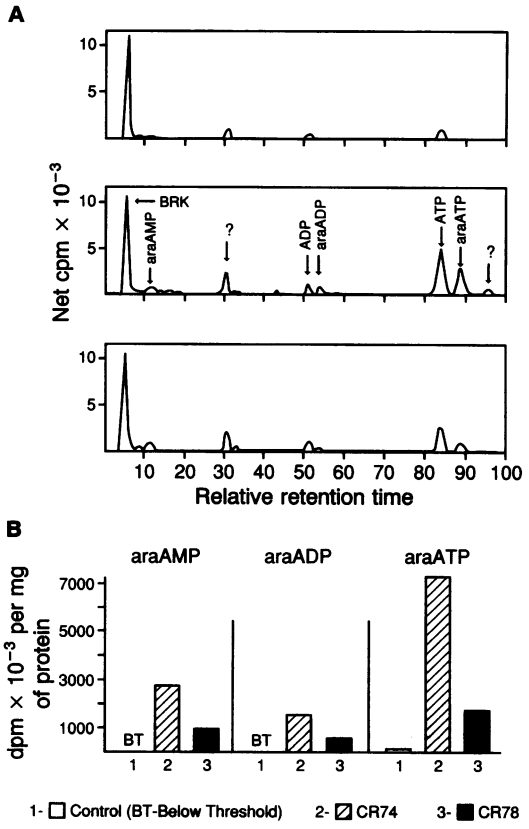


FIG. 4. Metabolism of araM. (*A*) Anabolism of araM in parental Hep G2 cells (control; top) and Hep G2 cells infected with CR74 (middle) and CR78 (bottom). BRK, breakthrough. (*B*) Relative anabolism of araM to araAMP, araADP, and araATP in parental Hep G2 cells (control) and Hep G2 cells infected with CR74 and CR78. Total recovery was based on the recovery of the internal standard, inosine triphosphate.

icant VZV TK activity experienced significantly increased sensitivity to araM compared with the parental cell controls. These data indicate that the VDEPT viruses, which generate tissue-specific expression of VZV TK and tissue-specific cytotoxicity from araM exposure, will phenotypically change those cells to become sensitive to araM.

**Selectivity of VZV TK Expression.** The data in Table 1 indicate that the nonhepatoma cell lines infected with the VDEPT viruses CR74 and CR78 did not experience any significant increase in either VZV TK activity or sensitivity to araM compared with their parental cell lines. It would be interesting, however, to examine individual cells in a G418-resistant population to determine if there are rare individual cells that exhibit significant VZV TK activity. NIH 3T3, which are TK negative ($TK^{-/-}$), were infected with CR74 or CR78 virus. Based on the viral titers, >50,000 individual cells were infected with each virus. Subsequent to infection and G418 selection, these cells were plated at a density of 100,000 cells per well and grown in the presence of G418, HAT, TFT, G418 plus HAT, or G418 plus TFT. Growth in the presence of HAT or TFT selects for cells that are phenotypically TK positive or TK negative, respectively. TFT is a substrate for VZV TK (G. Roberts and J. Fyfe, personal communication). Fig. 5 illustrates that control NIH 3T3 $TK^{-/-}$ cells did not grow in the presence of HAT but did grow in the presence of TFT, since they are TK negative. Cells infected with CR74 or CR78 virus grew in the presence of G418 and TFT but not in HAT. Any CR74- or CR78-infected cell that converted to a TK-positive phenotype would have resulted in a colony in HAT medium. These data illustrate that CR74 and CR78 viruses did not phenotypically change even 1 of the 100,000 cells plated to become TK positive.

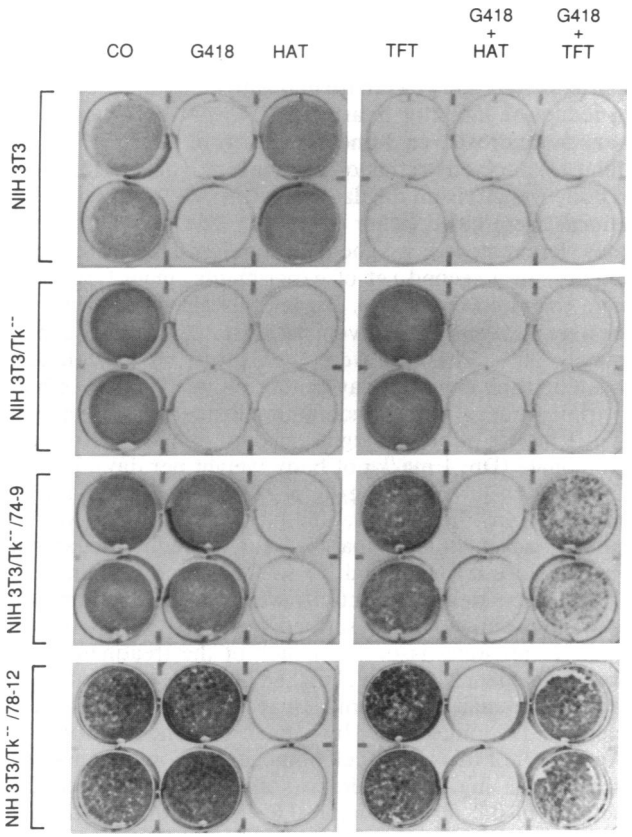


FIG. 5. Selectivity in NIH 3T3 $TK^{-/-}$ cells. Parental NIH 3T3 cells, TK-negative NIH 3T3 cells, and TK-negative NIH 3T3 cells infected with the VDEPT viruses CR74 and CR78 were grown in control medium (CO), G418, HAT, TFT, G418 plus HAT, or G418 plus TFT. Plates were stained using Giemsa's stain.

Taken collectively, these data suggest an innovative approach for the treatment of HCC involving retroviral-mediated gene therapy. Viruses were constructed that, subsequent to infection, selectively express the nonmammalian VZV TK enzyme in liver (CR74 virus) or more selectively in hepatoma cells (CR78). The cells that selectively express VZV TK become selectively sensitive to araM based on the selective anabolism of araM to araATP. There are two reasons to anticipate that both VDEPT viruses, CR74 and CR78, may be therapeutically effective in treating HCC *in situ*. (*i*) Since retroviruses require dividing cells to efficiently integrate, normal hepatocytes, which are essentially nondividing, should not be efficiently infected by these vectors. (*ii*) If a normal hepatocyte did integrate the ALB TRS/VZV TK chimera (CR74) and subsequently expressed VZV TK, the generation of araATP should be relatively nontoxic to the nondividing hepatocyte. The AFP TRS/VZV TK chimera (CR78) should exhibit an even greater degree of selectivity for hepatoma cells since VZV TK will only be expressed in the AFP-positive hepatoma cells.

This approach may open up new avenues for treatment of HCC as well as other types of cancers with transcriptionally-regulated marker genes. Such variations on this general theme may involve the treatment of certain colorectal tumors using carcinoembryonic antigen TRSs to regulate the expression of VZV TK. Based on recent clinical advances involving gene therapy, it may be feasible to attempt such approaches in human cancer patients in the future. Studies evaluating the utility of these viruses for the treatment of HCC *in situ* are required.

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