REVIEW

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Monitoring of gene transfer for cancer therapy with radioactive isotopes

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GENE THERAPY OF CANCER

THE EFFECTIVE TREATMENT of patients with malignant tumors is hampered by the similarity of normal and transformed cells. Local therapies as surgery and radiation therapy are only successful if the tumor cells are located to the area under treatment. In patients with disseminated disease a systemic treatment is necessary. However, although many different chemotherapeutic drugs are available and there has been considerable progress in the therapy of hematologic disease, the treatment of solid tumors is still a problem. The recent progress in basic science has delivered a better understanding of the mechanisms of carcinogenesis, tumor progression and the patients immune response towards the tumor. These developments are the conceptional and technological prerequisites for the development of new approaches for cancer treatment. The characterization of tumor cell-specific properties allows the design of new treatment modalities, which circumvent resistance mechanisms towards conventional chemotherapeutic drugs. Currently four approaches are evaluated in experimental and clinical studies: 1) protection of normal tissues (as the bone marrow), which are normally targets for cytotoxic drugs. This may be achieved by the transfer of the gene for glycoprotein p, a drug efflux pump. The presence of this pump in bone marrow cells may regulate the intracellular concentration of chemotherapeutic drugs in bone marrow progenitor cells at a lower non-toxic level and thereby protect these cells selectively. 2) improvement of the host antitumor response by increasing the antitumor activity of tumor infiltrating immune competent cells or by modifying the tumor cells to enhance their immunogeneity. This may be done by introduction of genes which are responsible for the production of foreign surface antigens and elicit a concomitant immune response against the foreign antigen and the otherwise unrecognized tumor antigen. This may result in the elimination of the genetically modified as well as the wild type tumor cells. The expression of cytokines in tumor-infiltrating lymphocytes or in the tumor cells leads to an enhanced intratumoral cytokine concentration which directs and activates immune competent cells in the tumor. 3) Reversion of the malignant phenotype either by suppression of oncogene expression or by introduction of normal tumor suppressor genes. The inactivation of oncoproteins may be performed by introduction of genes for intracellular antibodies (intrabodies) against these oncogenes or by the use of antisense oligonucleotides and ribozymes. 4) direct killing of tumor cells by the transfer of cytotoxic or prodrug-activating genes.

The transfer and expression of suicide genes into malignant tumor cells represents an attractive approach for human gene therapy. Suicide genes typically code for non-mammalian enzymes which convert nontoxic prodrugs into highly toxic metabolites. Therefore, systemic application of the nontoxic prodrug results in the production of the active drug at the tumor site. A broad range of suicide principles has been described (Table 1). The suicide systems which are applied in most studies are the cytosine deaminase (CD) and Herpes Simplex Virus thymidine kinase (HSV-tk).

Cytosine deaminase, which is expressed in yeasts and bacteria, but not in mammalian organisms converts the antifungal agent 5-fluorocytosine (5-FC) to the highly toxic 5-fluorouracil (5-FU). In mammalian cells no anabolic pathway is known which leads to incorporation of 5-FC into the nucleic acid fraction. Therefore pharmacological effects (which are due to conversion of 5-FC to 5-FU by the intestinal microflora) are moderate and allow the application of high therapeutic doses.[1,2] The distribution of 5-FC in mammalian tissues is uniform and neither

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Table 1

Enzyme	Prodrug	Active drug
E. coli DeoD	6-methylpurine-2′-deoxyribonucleoside	6-methylpurine
Xanthine oxidase	Xantine, hypoxanthine	H_2O, OH and O_2 radicals
Carboxypeptidase G2	Benzoic acid mustards-glumatic acid	Benzoic acid mustards
Alkaline phosphatase	Etoposide phosphate, doxorubicin phosphate, mitomycin phosphate	Etoposide, doxorubicin, mitomycin phenol mustard
Carboxypeptidase A	Methotrexate-alanine	Methotrexate
Cytosine deaminase	5-Fluorocytosine (5FC)	5-Fluorouracil (5FU)
Penicillin amidase	Doxorubicin-phenoxyacetamide Melphalan-phenoxyacetamide Palytoxin-4 Hydroxyphenoxyacetamide	Doxorubicin Melphalan Palytoxin
β-glucosidase	Amygdalin	Cyanide
β-glucoronidase	Epirubicin-glucoronide, phenol mustard-glucuronide, daunomycin-glucoronide, adriamycin-glucoronide	Epirubicin, phenol mustard, daunomycin, adriamycin
β-lactamase	Phenylenediamine mustard cephalosporin	Phenylenediamine mustard
nitroreductase	CB1954 (5-aziridin 2,4-dinitrobenzamidine)	5-Aziridin 2,4-hydroxyamino 2-nitrobenzamidine
HSV thymidine kinase	Ganciclovir (GCV), Aciclovir (AVC)	phosphorylated metabolites
VZV thymidine kinase	6-methoxypurine arabinonucleoside (araM)	phosphorylated metabolites

tissue-specific accumulation nor appreciable binding to plasma proteins have been observed.[2,3] 5-FU exerts its toxic effect by interfering with DNA and protein synthesis due to substitution of uracil by 5-FU in RNA and inhibition of thymidilate synthetase by 5-fluorodeoxy-uridine monophosphate resulting in impaired DNA biosynthesis.[4] Nishiyama et al.[5] implanted CD-containing capsules into rat gliomas and subsequently treated the animals by systemic application of 5-FC. They observed significant amounts of 5-FU in the tumors as well as a decrease in tumor growth rate and systemic cytotoxicity. This approach for local chemotherapy was expanded by Wallace et al.[6] for the application in patients with disseminated tumor disease. They used monoclonal antibody (mAb)-enzyme conjugates to achieve a selective activation of 5-FC thereby obtaining a 7-fold higher level of 5-FU in the tumor after administration of mAb-CD and 5-FC compared to the systemic application of 5-FU.

Gene therapy with herpes simplex virus thymidine kinase as suicide gene has been performed in a variety of tumor models *in vitro* as well as *in vivo*.[7–14] In contrast to human thymidine kinase HSV-tk is less specific and phosphorylates also nucleoside analogs such as acyclovir and ganciclovir (GCV) to their monophosphate metabolites.[15] These monophosphates are subsequently phosphorylated by cellular kinases to the di- and triphosphates. After integration of the triphosphate metabolites into DNA, chain termination occurs, followed by cell death.

Targeting the enzyme to the tumor site may be achieved by the transfer and expression of the suicide gene by use of recombinant retroviral vectors. Encouraging results have been initially obtained in rat gliomas using a retroviral vector system for transfer and expression of the HSV-tk gene.[13,14] Recently, *in vitro* and *in vivo* studies have further demonstrated the potency of the CD suicide system. Tumor cells which had been infected with a retrovirus carrying the cytosine deaminase gene showed a strict correlation between 5-FC sensitivity and CD enzyme activity.[16–18] However, although not all of the tumor cells have to be infected to obtain a sufficient therapeutic response, repeated injections of the recombinant retroviruses may be necessary to reach a therapeutic level of enzyme activity in the tumor. Therefore a prerequisite for gene therapy using a suicide system is monitoring of suicide gene expression in the tumor for two reasons: to decide if repeated gene transductions of the tumor are necessary and to find a therapeutic window of maximum gene expression and consecutive prodrug administration.[19] Since 5-FC as well as GCV can be labeled with ^{18}F with sufficient *in vivo* stability,[20,21] positron emission tomography (PET) may be applied to assess the enzyme activity *in vivo*.

Gene therapy using suicide genes is performed in two steps: first, the tumor is infected with recombinant viruses to introduce the suicide enzyme into the cells. To obtain a sufficient level of enzyme activity in the tumor multiple

infections may be necessary. Second, the nontoxic prodrug is applicated systemically. For the planning and the individualization of gene therapy the enzyme activity induced in the tumor has to be estimated in order to achieve a therapeutically sufficient enzyme level before the application of the prodrug. Moreover, the measurement of therapy effects on the tumor metabolism may be useful for the prediction of therapy outcome at an early stage of the treatment. Positron emission tomography (PET) using tracers of tumor metabolism has been applied for the evaluation of treatment response in a variety of tumors and therapeutic regimens,[22–25] indicating that these tracers deliver useful parameters for the early assessment of therapeutic efficacy.

MONITORING OF GENE THERAPY BY THE ASSESSMENT OF METABOLIC EFFECTS

Monitoring of gene therapy using imaging procedures for the assessment of morphological changes has been performed with magnetic resonance imaging techniques in rats bearing C6 rat glioblastomas and also in patients with glioblastoma.[26–28] In these studies, either marked tumor necrosis after interleukin-2 gene transfer or regression after induction of HSV-tk expression and GCV application were observed. Maron et al.[28] found an initial response to GCV treatment in 90% of the animals and a complete regression in two-thirds of the treated rats. Also, tumor recurrence could be observed. However, in these studies the therapeutic efficacy was evaluated using changes in tumor volume with examination intervals of two months between the end of the treatment and the first follow up examination.[27]

The measurement of metabolic changes after therapeutic intervention has proven to be superior to morphological procedures for the assessment of early therapy effects. In this respect, the FDG uptake has demonstrated to be a useful parameter for the evaluation of glucose metabolism.[23,24,29,30] Since the HSV-tk/GCV system induces DNA chain termination, we also expect changes in thymidine incorporation into tumor cell DNA occur. This may be assessed using [^{11}C]thymidine which has been applied to determine DNA synthesis *in vivo*.[31,32]

After transfection of a rat hepatoma cell line (Morris hepatoma MH3924A) with a retroviral vector containing the HSVtk gene[33,34] uptake measurements using thymidine (TdR), fluorodeoxyglucose (FDG), 3-*O*-methylglucose, AIB and methionine were performed in the presence of different concentrations of ganciclovir (GCV). In the HSVtk-expressing cell line an increased (up to 250%) thymidine uptake in the acid-soluble fraction and a decrease to 5.5% in the acid-insoluble fraction was found. The decrease of radioactivity in the nucleic acid fraction occurs early (4 h) after exposure of the cells to GCV and represents DNA chain termination induced by the HSVtk—ganciclovir system (Fig. 1). The phenomenon of a posttherapeutic increase of TdR or its metabolites in the acid-soluble fraction was observed in former studies after chemotherapy.[22] This effect may be explained by an increase in the activity of salvage pathway enzymes e.g. of host thymidine kinase activity during repair of cell damage. Therefore, PET measurements with ^{11}C-TdR may be used to assess the effects of the HSVtk-GCV system on DNA synthesis if quantitation is based on a modeling approach.

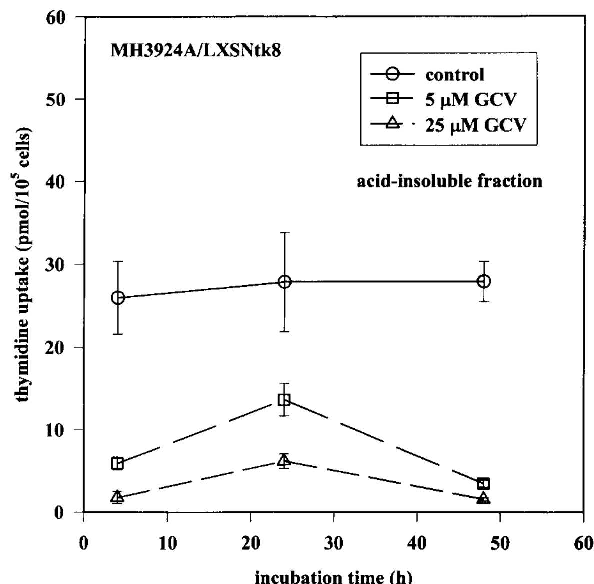

Fig. 1 Thymidine incorporation in HSVtk-expressing Morris hepatoma cells: uptake of ^{3}H-TdR in the acid-insoluble fraction in cells with or without treatment with ganciclovir (GCV).

During GCV treatment the uptake for FDG and 3-*O*-methylglucose increases up to 195% after 24 hours incubation with GCV. An HPLC analysis revealed a decline of the FDG-6-phosphate fraction after 48 hours incubation with GCV. Consequently, a normalization of FDG uptake was observed after this incubation period, whereas the 3-*O*-methylglucose uptake was still increased. Experiments performed with different amounts of HSVtk-expressing cells and control cells showed that these effects are dependent on the percentage of HSVtk-expressing cells.[33] The AIB uptake decreased to 47%, while the methionine uptake in the acid-insoluble fraction decreased to 17%.

In clinical and experimental studies an increase of FDG uptake early after treatment of malignant tumors has been described.[24,29,35,36] Cell culture experiments with rat adenocarcinoma cells under chemotherapy revealed that this effect is predominantly caused by an enhanced glucose transport.[35] As underlying mechanism a redistribution of the glucose transport protein from intracellular pools to the plasma membrane may be considered and is observed in cell culture studies as a general reaction to cellular stress.[37–40] Since prodrug activation by the HSVtk leads to DNA chain termination and cell damage, the same reactions may also occur in tumor cells under gene

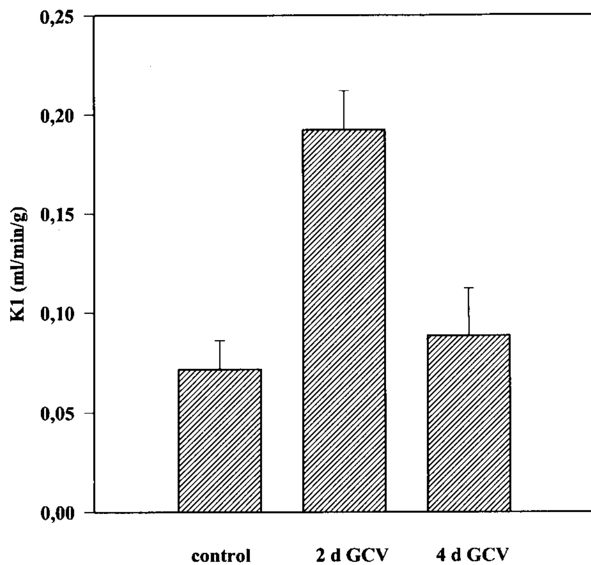

Fig. 2 FDG transport in untreated tumors (n = 8) and in tumors after 2 days (n = 7) or 4 days (n = 5) treatment with 100 mg GCV/kg body weight.

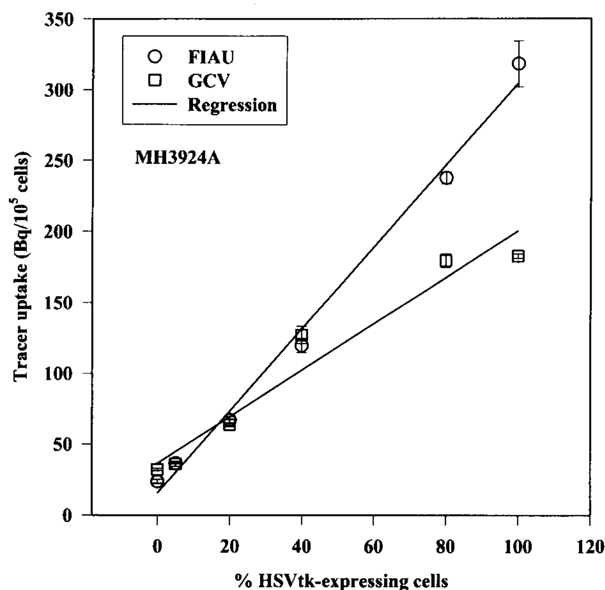

Fig. 3 GCV and FIAU uptake in different mixtures of control cells and HSVtk-expressing Morris hepatoma cells after 4 h incubation. The tracer uptake and the amount of HSVtk-expressing cells were correlated with r = 0.97 and r = 0.99, respectively.

therapy with this suicide system. Translocation of glucose transport proteins to the plasma membrane as a first reaction to cellular stress may cause enhancement of glucose transport and represents a short-term regulatory mechanism which acts independent of protein synthesis. Therefore, the increase in FDG and 3-*O*-methylglucose uptake *in vitro* is interpreted as stress reaction of the tumor cells. However, an uncoupling of transport and phosphorylation was observed after 48 hours incubation. The amino acid uptake experiments point to an inhibition of protein synthesis as well as of the neutral amino acid transport.

After transplantation of the same HSVtk-expressing Morris hepatoma cells into ACI rats, dynamic PET measurements of ^{18}FDG uptake were performed in animals two days (n = 7) and four days (n = 5) after the onset of therapy with 100 mg GCV/kg body weight as well as after administration of sodium chloride (n = 8). The arterial FDG plasma concentration was measured dynamically in an extracorporeal loop and the rate constants for FDG transport (K_1, k_2) and FDG phosphorylation (k_3) were calculated using a three-compartment model modified for heterogeneous tissues. Furthermore, the TdR incorporation into the tumor DNA was determined after *i.v.* administration of ^{3}H-TdR. An uncoupling of FDG transport and phosphorylation was found with enhanced K_1 (Fig. 2) and k_2 values and a normal k_3 value after two days of GCV treatment.[41] The increase in FDG transport normalized after four days whereas the phosphorylation rate increased. The TdR incorporation into the DNA of the tumors declined to 25% of the controls after 4 days of GCV treatment. These data indicate that PET with ^{18}FDG and ^{11}C-TdR may be applied for monitoring of gene therapy with the HSVtk/GCV suicide system. Increased transport rates are evidence of stress reactions early after therapy. The changes in the phosphorylation rate *in vivo* may be due to a more active form of the hexokinase and need further investigation. The measurement of TdR incorporation into the tumor DNA can be used as an indicator of therapy efficacy. The question of the time period between the onset of prodrug administration and the follow up PET studies has to be answered in clinical studies.

MONITORING OF GENE THERAPY BY THE UPTAKE OF SPECIFIC SUBSTRATES

Taking into account that the efficiency of viral vectors may vary one question remains to be solved: how can we decide when a therapeutically sufficient suicide enzyme activity has been achieved in the tumor? This question can be adressed by measuring the uptake of specific substrates for the suicide systems.

In the rat hepatoma model[33,34] uptake measurements were performed up to 48 hours in a HSVtk-expressing cell line and in a control cell line bearing the empty vector using 5-iodo-2′-fluoro-2′-deoxy-1-*β*-D-arabinofuranosyl-uracil (FIAU), fluorodeoxycytidine (FCdR) and ganciclovir.

The FCdR uptake was higher in the HSVtk-expressing cells with a maximum after 4 hours (12-fold and 3-fold higher in the acid-insoluble and acid-soluble fraction, respectively). After longer incubation periods the FCdR uptake declined. HPLC analysis showed a rapid and

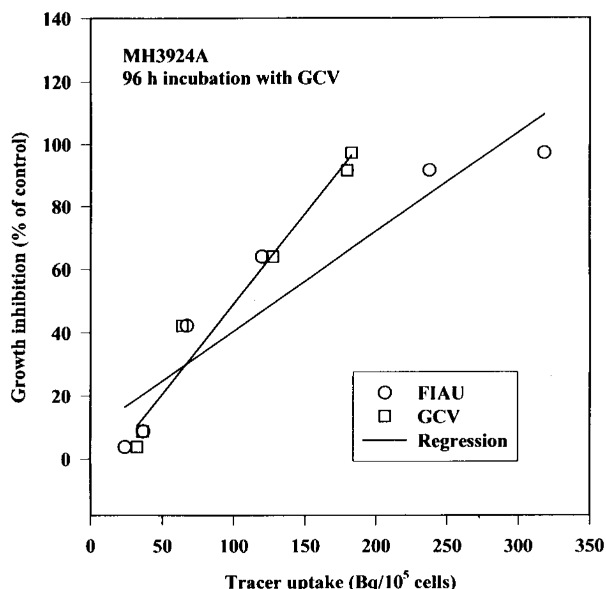

Fig. 4 Relation of total GCV and FIAU uptake after 4 h incubation and the growth inhibition after 24 hours and 48 hours exposure to 5 μM GCV. The tracer uptake and the growth inhibition in HSVtk-expressing Morris hepatoma cells were correlated with r = 0.98 and r = 0.94, respectively.

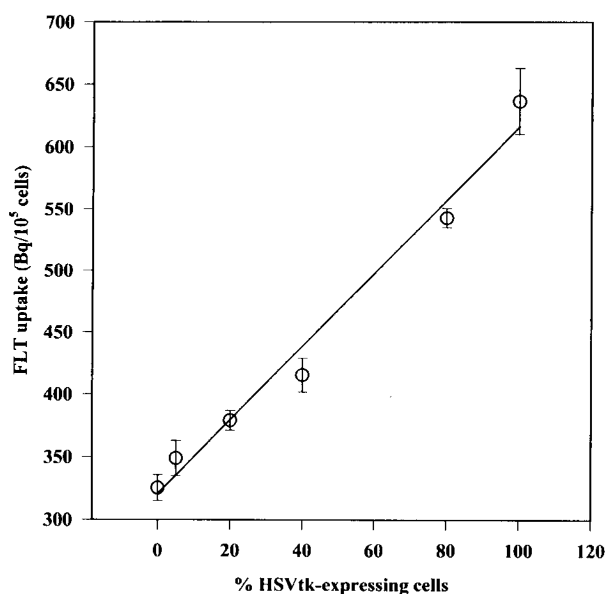

Fig. 5 FLT uptake in different mixtures of control cells and HSVtk-expressing Morris hepatoma cells after 4 h incubation. The tracer uptake and the amount of HSVtk-expressing cells were correlated with r = 0.98.

almost complete metabolization and degradation in both cell lines,[34] which might be due to dehalogenation or the action of nucleosidases. The GCV uptake showed a time-dependent increase in HSVtk-expressing cells and a plateau in control cells. The HPLC analysis revealed unmetabolized GCV in control cells and a time dependent shift of GCV to its phosphorylated metabolite in HSVtk-expressing cells.[34] Furthermore, the ganciclovir as well as the FIAU uptake were highly correlated to the percentage of HSVtk-expressing cells and to the growth inhibition as measured in bystander experiments[34] (Figs. 3 and 4). Similar results were obtained with genetically modified MCF7 human mammary carcinoma cells.[42] However, the rat Morris hepatoma cells revealed a much higher difference in GCV uptake than MCF7 cells, between HSVtk-expressing cells and control cells.[34] We also found that MCF7 cells are not as sensitive to the HSV-tk/GCV system as Morris hepatoma cells. This difference in the amount of tracer accumulation and sensitivity may be explained by the slower rate of growth of MCF7 cells (doubling time: 23.72 ± 2.4 h) as compared to Morris hepatoma cells (doubling time: 16.8 ± 0.7 h). Uptake studies with 3′-deoxy-3′-fluorothymidine (FLT), a new tracer for the assessment of thymidine kinase activity[58] also revealed a clear correlation of tracer uptake and the amount of HSVtk-expressing cells (Fig. 5). However, the difference in uptake was not as high as with GCV or FIAU, which is caused by the fact that FLT is a good substrate for the host thymidine kinase. This causes a high background due to the high thymidine kinase activity in tumor cells which is upregulated during the S phase in the cell cycle.

To further elucidate the transport mechanism of ganciclovir inhibition/competition experiments were performed. The nucleoside transport in mammalian cells is known to be heterogeneous. Two classes of nucleoside transporters have been described: the equilibrative, facilitated diffusion systems and the concentrative, sodium-dependent systems. In our experiments competition for all concentrative nucleoside transport systems and inhibition of the ganciclovir transport by the equilibrative transport systems was observed, whereas the pyrimidine nucleobase system showed no contribution to the ganciclovir uptake.[34,42] In human erythrocytes acyclovir has been shown to be transported mainly by the purine nucleobase carrier.[43] Due to a hydroxymethyl group on its side chain, ganciclovir has a stronger similarity to nucleosides and, therefore, may be transported also by a nucleoside transporter. Moreover, the 3′-hydroxyl moiety of nucleosides was shown to be important for their interaction with the nucleoside transporter.[44] However, in our experiments ganciclovir was mainly transported by the nucleoside transport systems.

In rat hepatoma cells as well as in human mammary carcinoma cells the GCV uptake was shown to be much lower than the thymidine uptake.[34,42] Therefore, in addition to low infection efficiency of the current viral delivery systems slow transport of the substrate and also its slow conversion into the phosphorylated metabolite is limiting for the therapeutic success of the HSV-tk/GCV system. Cotransfection with nucleoside transporters or the use of other substrates for HSV-tk with higher affinities for nucleoside transport and phosphorylation by HSV-

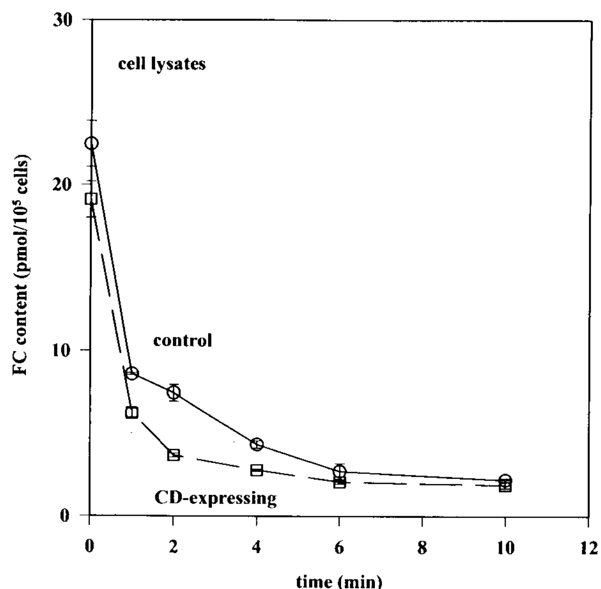

Fig. 6 5-FC content in cell lysates (pmol/10^5 viable cells) in CD-expressing human glioblastoma cells and in wild type cells after different incubation times in 5-FC-free medium. The cells were pulsed with ^{3}H-FC for 30 minutes prior to the efflux experiment.

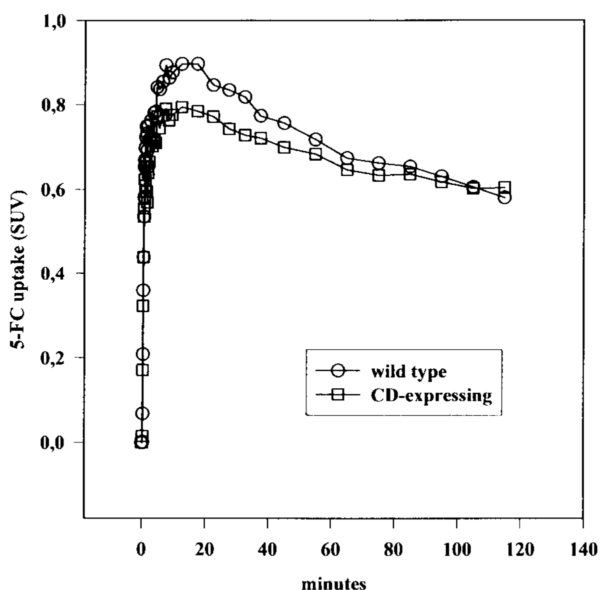

Fig. 7 Time-dependent accumulation of ^{18}F-fluorocytosine in Dunning prostate adenocarcinomas. Comparison between wild type tumors (n = 6) and tumors which were genetically modified to express the *E. coli* cytosine deaminase gene. No difference in tracer uptake could be observed.

tk is expected to improve therapy outcome.

The principle of *in vivo* HSVtk imaging was first demonstrated by Price et al. and Saito et al. for the visualization of HSV encephalitis.[45,46] Recently *in vivo* studies have been done by several groups using different tracers.[47–53] Gambhir et al. used 8-[^{18}F]fluoroganciclovir (FGCV) for the imaging of adenovirus-directed hepatic expression of the HSV1-tk reporter gene in living mice.[47] There was a significant positive correlation between the percent injected dose of FGCV retained per gram of liver and the levels of hepatic HSV1-tk reporter gene expression. Over a similar range of HSV1-tk expression *in vivo*, the percent injected dose retained per gram of liver was 0–23% for ganciclovir and 0–3% for FGCV. Alauddin et al.[48,49] used of 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)-guanine ([^{18}F]FHBG) and 9-[(3-^{18}F-fluoro-1-hydroxy-2-propoxy)methyl]-guanine ([^{18}F]-FHPG) for combined *in vitro*/*in vivo* studies with HT-29 human colon cancer cells, transduced with the retroviral vector G1Tk1SvNa. They also found a significant higher uptake in transduced cells as compared with the controls. *In vivo* studies in tumor-bearing nude mice demonstrated that the tumor uptake of the radiotracer is three and six-fold higher in 2 and 5 h, respectively, in transduced cells compared with the control cells. Others used radioiodinated nucleoside analogues as (E)-5-(2-iodovinyl)-2′-fluoro-2′-deoxyuridine (IVFRU) and 5-iodo-2′-fluoro-2′-deoxy-1-*β*-D-arabinofuranosyluracil (FIAU) to visualize HSVtk expression.[50–53] Tjuvajev et al.[52,53] used ^{131}I or ^{124}I labeled FIAU in brain and mammary tumors. Autoradiography as well as the SPECT and PET images revealed highly specific localization of the tracer to areas of HSV1-tk gene expression at 24, 36, and 48 h after i.v. administration. The amount of tracer uptake in the tumors was correlated to the *in vitro* ganciclovir sensitivity of the cell lines which were transplanted in these animals.[52,53] Wiebe et al.[50,51] report that IVFRU becomes metabolically trapped in tumor cells transduced with the HSVtk gene on a retroviral vector. Selective phosphorylation of radiolabeled IVFRU by HSVtk results in elevated radioactivity in HSVtk-expressing tumor cells *in vitro* and *in vivo* relative to cells lacking the HSVtk gene. Due to low non-target tissue uptake, unambiguous imaging of HSVtk-expressing tumors in mice is possible with labeled IVFRU. The advantage of iodinated tracers like FIAU may be that delayed imaging is possible. Since ^{18}F labeled compounds allow only imaging early after administration of the tracer these iodinated compounds may prove to be more sensitive *in vivo*. However, quantification with iodine isotopes may be a problem either with ^{131}I, a γ- and β^- emitter with high radiation dose, or with the corresponding positron emitter ^{124}I, which shows only 23% β^+ radiation with high energy particles, multiple γ rays of high energy and leads to a high radiation dose.

In human glioblastoma cells another suicide system, the cytosine deaminase (CD), was evaluated. CD expressing cells are able to convert the nontoxic prodrug 5-fluorocytosine (5-FC) to the toxic metabolite 5-fluorouracil (5-FU). Positron emission tomography (PET) with 5-(^{18}F)FC may be applied for the *in vivo* measurement of CD activity in genetically modified tumors. A human

glioblastoma cell line was stably transfected with the *E. coli* CD gene[19] and experiments with ^{3}H-FC were performed. ^{3}H-5-FU was produced in CD-expressing cells, whereas in the control cells only ^{3}H-5-FC was detected.[19] Moreover, significant amounts of 5-FU were found in the medium of cultured cells, which may account for the bystander effect observed in previous experiments. However, uptake studies revealed a moderate and nonsaturable accumulation of radioactivity in the tumor cells suggesting that 5-FC enters the cells only via diffusion.[19] Although a significant difference in 5-FC uptake was seen between CD-positive cells and controls after 48 hours incubation, no difference was observed after 2 hours incubation. Furthermore, a rapid efflux could be demonstrated (Fig. 6). Therefore, 5-FC transport may be a limiting factor for this therapeutic procedure and quantitation with PET has to rely rather on dynamic studies and modeling, including HPLC analysis of the plasma, than on nonmodeling approaches.[19]

To evaluate the 5-FC uptake *in vivo*, we transfected a rat prostate adenocarcinoma cell line with a retroviral vector bearing the E. coli CD gene. The cells were found to be sensitive to 5-FC exposure, but lost this sensitivity with time. This may be due to inactivation of the viral promoter (CMV) used in this vector. *In vivo* studies with PET and ^{18}FC showed no preferential accumulation of the tracer in CD-expressing tumors although HPLC analysis revealed a production of 5-fluorouracil which was detectable in tumor lysates as well as in the blood of the animals (Fig. 7).

NON-SUICIDE REPORTER GENE APPROACHES

The dopamine D2 receptor gene has also been used as a reporter gene.[54] This gene represents an endogenous gene which is not likely to invoke an immune response. Furthermore, the corresponding tracer 3-(2′-[^{18}F]-fluoroethyl) spiperone (FESP) rapidly crosses the blood-brain-barrier, can be produced at high specific activity and is currently used in patients. As a SPECT tracer [^{123}I]-Iodobenzamine is available. MacLaren et al. used this system in nude mice with an adenoviral-directed hepatic gene delivery system and also in stably transfected tumor cells which were transplanted in animals. The tracer uptake in these animals was proportional to *in vitro* data of hepatic FESP accumulation, dopamin receptor ligand binding and the D2 receptor mRNA. Also tumors modified to express the D2 receptor retained significantly more FESP than wild type tumors.

Another approach is based on the *in vivo* transchelation of oxotechnetate to a polypeptide motif from a biocompatible complex with a higher dissociation constant than that of a diglycilcysteine complex. It has been shown that synthetic peptides and recombinant proteins like the modified green fluorescence protein (gfp) can bind oxotechnetate with high efficiency.[55,56] In these experiments rats were injected i.m. with synthetic peptides bearing a GGC motif. One hour later ^{99m}Tc-glucoheptonate was applied i.v. and the accumulation was measured by scintigraphy. The peptides with three metal-binding GGC motifs showed a three-fold higher accumulation as compared to the controls. This principle can also be applied to recombinant proteins which appear at the plasma membrane. These genes can be cloned into bicistronic vectors which allow for the co-expression of therapeutic genes and *in vivo* reporter genes. Thereafter, radionuclide imaging may be used to detect gene expression.

Tyrosinase catalyzes the hydroxilation of tyrosine to DOPA and the oxidation of DOPA to DOPAquinone which after cyclization and polymerization results in melanin production. Melanins are scavengers of metal ions as iron and indium through ionic binding. Tyrosinase transfer leads to the production of melanins in a variety of cells. This may be used for imaging with NMR or with ^{111}In and a gamma camera. Cells transfected with the tyrosinase gene stained positively for melanin and had a higher ^{111}In binding capacity than the wild type cells.[57] In transfection experiments a dependence of tracer accumulation on the amount of the vector used could be observed. The problem of this approach are possible low tyrosinase induction with low amounts of melanin and the cytotoxicity of melanin. These problems may be encountered by the construction of chimeric tyrosinase proteins and by positioning of the enzyme at the outer side of the membrane.

Recently the rat sodium/iodide symporter gene (rNIS) was cloned into a retroviral vector for transfer into melanoma cells.[59] *In vitro* iodide transport experiments revealed that the symporter functions similarly in rNIS-transduced tumor cells as in rat thyroid follicular cells. rNIS-transduced and wild type human A375 melanoma xenografts transplanted into nude mice were imaged using a gamma camera after i.p. injections of ^{123}I and were visually distinguishable from and accumulate significantly more radionuclides than wilr type tumors.

FUTURE STUDIES

Future studies will be influenced by the development of new viral vectors with have to be more efficient in terms of infection rate and tissue specificity. Furthermore, the use of mutants of HSVtk may result in better imaging characteristics. Transfer of nucleoside transporters can be used to enhance the tracer influx into the genetically modified cells and thereby increase suicide enzyme detection as well as therapeutic efficiency. Receptor genes with a lower background as the dopamine D4 receptor or genes which allow the use of commercially available ligands as the somatostatin receptor subtype 2 may be evaluated for their use as *in vivo* reporter genes or even for therapeutic purposes.

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