Review Article

Suicide Gene Therapy against Cancer

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Abstract

A major limitation of conventional chemotherapies used in cancer treatments today are low therapeutic indices and side effects that result from drug effects on normal tissues (off target). One of the most innovative approaches to developing antineoplastic agents with increased tumor selectivity is the use of suicide gene therapy. Suicide gene therapy involves delivering a gene product in proximity to the targeted cancer tissue through various targeted delivery methods followed by tissue/tumor-specific expression of the gene product which then converts a systemically available pro-drug into an active drug within the tumor locale. Here we summarize the concept of gene therapy for cancer and introduce the most frequently used suicide gene therapy systems. In addition we discuss viral, molecular and cellular vectors and their advantages and disadvantages. Finally, we describe the clinical applications, limitations and potential side effects of suicide gene therapy to date.

Introduction

Cancer and its treatment are an immense burden not only on the individual patient but also on the entire healthcare system. Over 1.5 million patients are diagnosed with new cancer in the United States and more than 500,000 patients die of cancer each year [1]. Despite some improvements in outcomes of cancer patients, the high rate of cancer related deaths and overall rising incidence of cancer highlights some of the limitations of conventional cancer treatment strategies such as chemotherapy, radiotherapy and surgical resection. A more effective, patient tailored and innovative approach to targeting cancers is warranted.

Chemotherapy has long been the cornerstone of cancer treatment [2]. However, an important drawback of conventional chemotherapy is the relatively low therapeutic index due to the lack of tumor specificity. This has implications for chemotherapy dosing where the efficacy of a traditional chemotherapeutic agent must be balanced with toxicity related side effects. To overcome these limitations of conventional chemotherapy, newer approaches to increase treatment selectivity against cancer cells must be developed. One of these is the application of selective suicide gene therapy also known as Gene Directed Enzyme Prodrug Therapy (GDEPT) to cancer therapy.

The fundamental concept underlying suicide gene therapy is as follows: a gene is selectively introduced into the tumor environment which encodes for an enzyme that metabolizes a systemically available pro-drug to an active anti-neoplastic agent locally. Moolten et al. provided the first example of suicide gene approach for therapy of cancer in 1986, describing the introduction of the herpes simplex virus thymidine kinase gene into neoplastic BALB/c murine K3T3 sarcoma cell lines [3]. Treatment with ganciclovir, which is converted by thymidine kinase into compounds that become toxic after triphosphorylation by cellular kinases, resulted in destruction of the tumor cells in vitro. Administration of ganciclovir to BALB/c mice bearing K3T3 sarcoma tumors produced by the cell lines resulted in destruction of the tumors in vivo [3]. Since then the interest in cancer gene therapy has increased dramatically (Figure 1). The central rationale for suicide gene therapy is to artificially generate exploitable biochemical differences between healthy host tissues and cancer cells. Targeting of the cancer environment is achieved by selection of the vector used to deliver the suicide gene, as well as by the biology of

suicide gene / produg system employed. As a result, high doses of the drug generated only in the tumor environment result in limited side effects in other tissues. Suicide gene therapy of cancer also has further benefits in the form of bystander killing. This refers to the destruction of tumor cells that are not directly expressing the suicide gene. Gap junctions play a central role in mediating these effects. Gap junctions consist of intercellular channels that generate specialized intercellular connections between cells that are in direct contact. They facilitate a direct communication between the cytoplasm of two or more cells and the direct transfer of ions and small molecules between adjacent cells. The gap junction channel proteins belong to the connexin family that is expressed in almost all tissues. In suicide gene therapy, gap junctions allow local diffusion of the active drug resulting in an enhanced effect. In addition, a bystander effect can be mediated through the enhanced immune response as an effect of the resulting cancer necrosis which can sensitize the immune system independently from the expressed suicide gene.
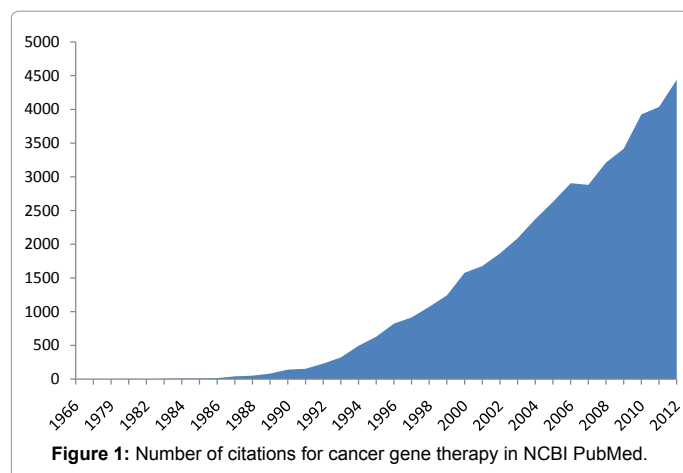
Bystander effects have been looked at in various in vivo models. For example, in a murine model of tumor xenografts expressing the cytosine deaminase suicide gene (see below), significant regression in all tumors was observed after dosing the prodrug 5-fluorocytosine even when only 2% of the tumor mass expressed the suicide gene [4]. In a second example, mice that were innoculated with a colon carcinoma cell line carrying the Uracil Phosphoribosyltransferase (UPRT) suicide gene responded to prodrug treatment and eliminated wild-type colon carcinoma cells of the same cell line, but not syngeneic tumor cells. This effect was not seen in nude mice, suggesting a role for the adaptive immune system in the bystander effect [5].

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Figure 1: Number of citations for cancer gene therapy in NCBI PubMed.

The goal of this review is to summarize the various concepts and experimental results underlying the developing field of suicide gene therapy of cancer, and as well as its clinical relevance as a therapeutic modality. This article will also briefly touch upon the limitations of suicide gene therapy.

Suicide Gene Systems for Cancer Therapy

Suicide gene therapy is based on the introduction of genes into tumor tissue which ultimately results in cancer cell death. The suicide gene/prodrug combination should ideally meet the following requirements [6]. First, the suicide gene should not be expressed in significant quantities in healthy organs. Second, it should specifically and efficiently catalyze the conversion of the chosen prodrug into the active anti-cancer agent. Finally, the suicide gene should fully activate the prodrug without the need for many additional endogenous enzymes (that may be mutated or not expressed by some tumors).

Similarly, there are specific requirements for the prodrug [6]. First, the prodrug should have minimal toxicity prior to activation. Second, it should have maximal toxicity for the cancer cells after conversion into the active drug. Third, it should be specifically converted into an active drug by the enzyme encoded by the suicide gene - but not other native enzymes outside the cancer. Fourth, the prodrug should be able to reach all cancer cells. A number of suicide gene systems have been developed, each with its own strengths and weakness with respect to the aforementioned characteristics (Table 1). The most important systems are discussed in details below.

Herpes Simplex Virus Thymidine Kinase/Gancyclovir

The most intensively studied suicide gene system is Herpes Simplex Virus Thymidine Kinase (HSV TK) and the prodrug ganciclovir [7]. HSV TK catalyzes phosphorylation of gancyclovir (GCV) to ganciclovir monophosphate [8]. Ganciclovir monophosphate can then be converted to di- and triphosphate derivatives by cellular kinases. The resulting compounds are toxic as they are incorporated by cellular DNA polymerases into DNA, resulting in DNA chain termination and apoptosis [7]. This system has been evaluated in a series of pre-clinical in vivo models that has lead to its application in various clinical trials for cancers such as glioblastoma and prostate cancer, which we will detail later in this article [9-12].

The limitations of the HSV/Tk-GCV system include potential immunogenicity of the viral enzyme, and the requirement for active mitosis for this system to induce cell death [13]. Moreover, GCV-

triphosphate enters cells passively or via gap junctions which can potentially limit overall therapeutic effects [14]. Other research approaches have led to the design of suicide gene therapy systems that try to circumvent these limitations.

Cytosine Deaminase/5-FU

The cytosine deaminase system was originally described as a negative selection system for experimental studies, and in treatments employing gene transfer techniques [15]. Cytosine deaminase is expressed by bacteria and yeast but is absent in mammalian cells. It normally catalyzes the deamination of cytosine to uracil and ammonia. However, this enzyme can also convert the prodrug 5-fluorocytosine (5-FC) to 5-Fluoro Uracil (5-FU), an important drug used in conventional chemotherapy of cancer. 5-FU enters the nucleotide salvage pathway and is processed to 5-Fluoro-2'-deoxyuridine-5'-Monophosphate (5-FdUMP), 5-Fluorouridine-Diphosphate (5-FUDP) and 5-Fluorouridine-Triphosphate (5-FUTP). 5-FdUMP is an irreversible inhibitor of thymidylate synthase, resulting in thymidine starvation and inhibition of DNA synthesis. 5-FUDP is also further processed to 5-FdUTP, which can be incorporated into DNA and lead to DNA damage and apoptosis. 5-FUTP can also be incorporated into RNA, substituting for UTP and inhibiting RNA processing [6]. The suicide gene therapy achieved in this case minimizes the normal systemic side effects of 5-FU therapy, and maximizes the potential local anti-tumor effect. Success in preclinical animal models has led to expanded clinical trial applications in breast and prostate cancer [9-11].

Carboxyl Esterase/Irinotecan (CE/CPT-11)

Carboxyl esterase is a serine esterase found in a variety of tissues, including serum, liver and the intestine [16]. Carboxyl esterase can enhance the general bio-availability of various therapeutic agents. For example, the Chemotherapy Prodrug Irinotecan (or CPT-11) is cleaved by the enzyme to generate the potent anti-tumor agent 7-ethyl-10-hydroxycamptothecin (SN-38), an inhibitor of topoisomerase I activity [17]. SN-38 is insoluble and therefore only acts locally. Inhibition of topoisomerase I by SN-38 lead to the accumulation of double-strand DNA breaks in actively dividing cancer cells resulting in inhibition of both DNA replication and transcription. In a pre-clinical study, an adenovirus vector carrying the carboxyl esterase gene was injected directly into subcutaneous human lung A549 carcinoma cell lines in mice. In mice receiving CPT-11, this resulted in a 35% reduction in tumor size after 27 days [18].

Varicella Zoster Virus Thymidine Kinase/6-Methoxypurine Arabinonucleoside (Vzvtk/Aram)

The Varicella Zoster virus also encodes for a Thymidine Kinase (VZV-TK) that is responsible for the activation of the antiherpetic nucleoside acyclovir. VZV-TK was one of the first suicide genes evaluated for the genetic modification of tumor cells. 6-methoxypurine Arabinonucleoside (ara-M) acts as a prodrug for the VZV-TK enzyme. Ara-M is monophosphorylated by VZV-TK leading to Ara-M Monophosphate (ara-MMP). It is then further metabolized to a highly toxic adenine Arabinonucleoside Triphosphate (ara-ATP) by four cellular enzymes: AMP deaminase, adenylosuccinate synthetase lyase, AMP kinase, and nucleoside diphosphate kinase. One problem associated with the VZV-TK suicide gene system is the generally poor efficacy of the activated prodrug against some cancer cell types. Various antiherpetic pyrimidine nucleoside analogs have been recently developed which show improved potency. For example, the novel prodrug (E)-5-(2-bromovinyl)-2-deoxyuridine (BVDU) was found to

Suicide Gene Product	Prodrug	Drug
Herpes simplex virus thymidine kinase	Ganciclovir	Ganciclovir triphosphate
Varicella-Zoster virus thymidine kinase	6-Methoxypurine arabinoside	Adenine arabinoside triphosphate
Cytosine deaminase	5-Fluorocytosine	5-Fluorouracil
Purine nucleoside phosphorylase	6-Methylpurine-2-deoxyriboside	6-Methylpurine
Nitroreductase	5-Aziridinyl-2,4-dinitrobenzamide	5-(Aziridinyl)-4-hidroxylamine-2-nitrobenzamide
Beta-Galatosidase	N-[4″-(beta-D-galactopyranosyl)-3″-nitrobenzyloxycarbonyl] daunomycin	Daunomycin
Hepatic cytochrome P450-2B1	Cyclophosphamide and Ifosfamide	Phosphoramide mustard and acrolein
Linamarase	Linamarin	Cyanide
Horseradish peroxidase	Horseradish Indole-3-acetic acid and derivatives, paracetamol	Free radicals
Carboxypeptidase A	Methotrexate (MTX)-α-peptides	MTX
Carboxypeptidase G2	N,N-[(2-chloroethyl) (2-mesyloxy-ethyl) amino] benzoyl-L-glutamic acid	N,N-[(2-chloroethyl) (2-mesyloxyethyl) amino] benzoic acid

Table 1: Suicide Gene Systems.

be 80 fold more toxic to cells expressing the VZV-TK transgene than the ara-M compound.

Nitroreductase Nfsb/ 5-(Aziridin-1-Yl)-2 4-Dinitro-benzamide (NTR/CB1954)

Nitroreductase NfsB (NTR) is a flavoprotein derived from Escherichia coli that shows broad substrate specificity. The enzyme acts by reducing quinones and nitroaromatics via NADPH or NADH. The prodrug 5-(aziridin-1-yl)-2 4-dinitrobenzamide (CB1954) is reduced by this enzyme leading to the production of a potent cytotoxic agent. This can lead to cell cycle independent DNA interstrand crosslinking [19]. Anti-cancer agents that do not target the cell cycle are important for effectively treating slow growing cancers such as prostate cancer and liver cancer. This agent is also cell-permeable resulting in an efficient bystander effect [20]. The NRT/CB1954 system was used clinically in prostate cancer. Patients with non-metastatic prostate cancer who had failed local treatment were treated with direct intraprostratic injection of an adenoviral vector encoding nitroreductase and subsequently treated systemically with the prodrug CB1954. This phase I/II clinical trial showed minimal toxicity of the systemic prodrug or the vector. There was also a delay in progression of the tumor marker PSA at 6 months [11].

Carboxypeptidase G2/4-[(2-chloroethyl)(2-mesyloxy-ethyl)amino]benzoyl-L-glutamic acid (CPG2/CMDA)

The bacterial enzyme Carboxypeptidase G2 (CPG2) cleaves the glutamic acid moiety from the prodrug 4-[(2-chloroethyl) (2-mesyloxyethyl) amino]benzoyl-L-glutamic acid (CMDA). This leads to production of a DNA-cross-linking mustard drug 4-[(2-chloroethyl) (2-mesyloxyethyl) amino] benzoic acid which does not require additional metabolic modification to act as a potent cytotoxic agent. While most cells expressing CPG2 are sensitive to CMDA, some tumors are resistant to this toxin due to limited uptake of the prodrug. To help address this, mutants of the CPG2 gene have been generated that result in targeting of the CPG2 protein to the cell membrane and expression as a cell surface protein. This modification of the suicide gene CPG2 leads to increased toxicity of the prodrug CMDA in previously resistant cells, and a very efficient bystander effect.

The suicide gene approach encompasses a diverse series of potential therapeutic systems. Despite its advantages, suicide gene therapy as cancer therapy can be limited by suboptimal delivery of the suicide gene to target tissues. A major focus of GDEPT/suicide gene research is establishing specific and efficient methods of delivery the therapeutic gene to the tumor environment.

Targeted Delivery of Suicide Genes

Once a suitable suicide gene system has been selected, the suicide gene must be selectively delivered to the cancer environment. The ideal delivery vehicle should have minimal side-effects, show specificity for the cancer environment, and achieve efficient gene delivery to the target cells. A number of delivery methods have been described for the selective delivery of suitable genes into the local environment of the cancer cells [21]. These general delivery methods can be classified into the following categories: viral vectors, molecular vectors and cellular vectors.

Viral vectors

Viral- based vectors are an efficient means for the delivery of suicide genes into cancer cells. Due to their high transduction efficiency, they are the most frequently used method to date for gene delivery strategies in clinical gene therapy trials [22,23]. Viral vectors are designed by introducing therapeutic genes into a well characterized set of viral elements. The choice of the viral system employed depends upon the tissue target and suicide gene system employed.

Adenoviruses are the most popular viral vectors used to date in clinical trials because of their ability to transduce both dividing and non-dividing cells [22]. This is a non-enveloped DNA virus with a 36 kb genome structured into an early region (E1 through E4), two delayed early units (IX and 1Va2), a late region (L1 through L5) and VA regions. This genome is flanked by two short-inverted terminal repeat sequences. Adenoviruses enter the target cell by receptor-mediated endocytosis and are transported to the nucleus. Subsequently the viral genes are transcribed and gene products expressed by cellular enzymes. Adenovirus vectors are designed by replacing viral sequences in adenovirus DNA by cDNA for the suicide gene. This results in high-level gene expression. However, the major disadvantage of this approach is that gene expression is transient as adenovirus does not integrate into the target cell genome. New methods are under development to generate hybrid adeno-based vectors with the capacity to integrate into the genome of target cells. A challenge of adenoviral based gene therapy is the intensive immune reaction elicited by the vector which has led to fatal reactions in some early patient trials. Furthermore, an adenovirus based gene replacement therapy trial of ornithine transcarbamylase deficiency resulted in the death of a study patient due to anaphylactic shock in response to the immunogenic adenovirus [24].

Retroviral vectors have also been employed for gene delivery in some clinical trials [25]. For example, the first successful gene therapy trial, which corrected x-linked severe combined immunodeficiency by inserting the gene for the common gamma chain into hematopoietic

stem cells, employed a retroviral vector [26,27]. One of the major advantages of using retroviruses is their ability to stably integrate into the target cell genome. Retroviruses have RNA genomes flanked by terminal repeat sequences (LTR). The genome includes genes for ribonucleoprotein core (gag), protease reverse transcriptase and integrase enzymes (pol) and envelope glycoproteins (env). For suicide gene therapy, these genes can be replaced with a promoter driving a cDNA for the desired suicide gene. In this case, cell lines which express gag, pol and env can provide the necessary proteins to complete the viral particle [28]. Following attachment of the viral particle to the cell, the RNA is reverse transcribed into DNA which is then integrated into the host genome. In the lentivirus subclass of retroviruses, this integration can be achieved in dividing as well as non-dividing cells. One important concern in the use of retroviral vectors is the size limitations for the transgene package. Clinical trials performed using viral vectors have also highlighted some of the difficulties in terms of the safety and toxicity of these vectors when used directly to treat genetic disease [22]. For example, retroviral vectors can result in insertional mutagenesis which represents the major drawback of these vector systems [29]. This became drastically evident in a clinical trial of gene therapy for x-linked severe combined immunodeficiency, in which two out of 10 patients developed leukemia due to retroviral integration in proximity to the LMO2 proto-oncogene promotor [30]. Clearly, caution should be exercised when using viral vectors for the treatment of human diseases; however, this becomes less of an issue when these vectors are used to engineer cellular vehicles ex vivo effectively reducing toxicity and safety issues, a topic that will be addressed later in this review.

Molecular vectors

The various problems associated with the use of viral vectors have led to an increased focus on non-viral systems for the delivery of genetic materials to tumor environments. These methods include the use of various non-viral vectors, as well as physical approaches that result in direct gene transfer. The most basic molecular vector is naked DNA. This approach relies on the non-specific uptake of the DNA by cancer cells, a process that is relatively inefficient. This approach is suitable if a relatively low proportion of successfully introduced genes are sufficient to provide a therapeutic effect. The efficiency of this approach can be increased by various physical methods, such as the use of a gene gun. Another physical method of gene delivery is the use of hydrodynamic gene transfer. This technique relies on controlled hydrodynamic pressure application into capillaries in order to increase cellular permeability. As a result, solutions containing naked DNA can be delivered more effectively [31].

DNA may also be combined with vehicles to enhance uptake [32]. These non-viral vector systems are relatively safe, can be easily constructed and exhibit high gene encapsulation ability. Non viral approaches include the application of cationic polymers such as polyethylenimine or poly-L-lysine, cationic peptides, and cationic liposomes. Among these vectors, the liposome approach has been the most widely applied in clinical trials for tumor therapy [33]. Gene delivery using cationic liposomes was first demonstrated using a synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA). Small unilamellar liposomes containing DOTMA interact spontaneously with DNA to form lipid-DNA complexes. Subsequently, DOTMA will facilitate fusion of the DNA containing complex with the plasma membrane of target cells, resulting in both uptake and expression of the DNA [32]. To enhance targeting, another molecular vector strategy relies on the incorporation of single chain variable fragment (scFv) antibodies against tumor proteins allowing an active targeting of tumor cells. Therapeutic transgenes have been successfully delivered into ovarian cancer cells using scFV antibodies directed against HER2/ neu, RON, and NK1R, [34]. An important advantage of these vectors is that they are considered to be safer than viral vectors because of the reduced chance for insertional mutagenesis or side effects from immune reactions to the vector [35].

Shell nanoparticles represent a novel form of gene transfer vehicles. These nanoparticals include a cationic core. This approach offers the option of high gene transfection efficiency with potential delivery of drugs and genes to the same cells. This approach can make use of passive or active cell targeting.

Cellular vector

Mesenchymal Stem Cells (MSC) are pluripotent progenitor cells that contribute to the maintenance and regeneration of diverse tissues after injury and during chronic inflammation [36]. The damaged tissue releases signals which result in the mobilization of MSCs and their subsequent recruitment to the site of injury. Tumors are seen by the body as something akin to a chronic wound, and as a result, MSCs are actively recruited to the tumor environment [37]. MSCs can contribute to diverse aspects of the tumor niche by acting as progenitor cells for tumor vessels and stromal-fibroblast-like cells [38]. The exact mechanism of this homing is still unclear, but likely involves chemokine biology [39] vascular endothelial growth factor (VEGF) [40], in the initial homing - as well as the cellular adhesion molecules integrin a4B1, vascular cell adhesion molecule 1 and cellular fibronectin in diapedesis and transmigration [39,41,42].

These cells represent promising delivery vectors for suicide gene therapy of cancer. MSCs can be isolated by conventional means (e.g. bone marrow isolation), are accessible for genetic modification in vitro, and can be readily expanded in culture. MSCs successfully engraft into tissues under conditions of increased cell turnover, such as seen during neoplastic growth. MSCs are also largely immune privileged as they lack expression of MHC class II, show low expression of MHC class I, and lack expression of CD40, CD80 and CD86. This also suggests that the use of allogeneic MSCs may not be problematic in patent settings.

A series of groups have shown that MSCs modified with suicide genes (e.g. HSV-TK) retain tumor tropism [43]. The cells are successfully recruited to various experimental tumors types and elicit anti-tumor effects in combination with GCV. Importantly, genetically modified MSCs bypass the need for myeloablation and bone marrow transplantation. They retain their stem cell features and homing capacity and maintain long-term transgene expression in vitro and in vivo. In a study performed by Hung et al., human MSCs which had been transduced using lentiviral vectors to express HSV-TK efficiently homed to subcutaneous colon cancer in a murine model [44]. Matuskova et al. also introduced the HSV- TK gene into human MSCs using retroviral vectors, and showed that after i.v. injection the transduced human MSCs homed to a subcutaneous glioma in a murine model, and led to therapeutic effect following GCV treatment [45]. Song et al. transduced rat MSCs with a lentiviral vector expressing HSV-TK and successfully treated subcutaneously growing prostate cancer in a rat model [46]. In most of these tumor models, treatment with the engineered MSCs showed substantial and efficient homing to tumors, as well as efficacy regarding inhibition of local tumor growth, suppression of metastasis, or prolongation of animal survival.

Tissue Specific Promoters Allow Selective Delivery of Therapeutic Gene Expression

While MSCs appear to be relatively efficient in their migration to tumor environments, they also clearly migrate to non-tumor tissues and sequester in lung [47]. Tissue specific promoters linked to the differentiation pathway initiated in the recruited stem cell within tumor environments have been employed to help restrict the expression of suicide gene to tumor environments. To this end, MSC have been engineered to express therapeutic transgenes when the cells are recruited to tumor tissue and become activated by tumor-specific signals. These selective effects depend upon specific gene promoters that are induced when the MSC undergo selective activation or specific differentiation programs. This approach allows the selective expression of therapeutic genes only within a defined biologic context. MSCs that are recruited to other tissue niches, but do not undergo the same program of differentiation or activation, do not induce expression of the therapeutic gene. This approach has led to a significant degree of control for the selective expression of the therapeutic genes within a defined microenvironment for example; tumor angiogenesis or the development of tumor stromal fibroblasts.

We have shown the promise of this therapeutic approach in proof-of-principle experiments using two in vivo models. The first used a syngeneic orthotopic pancreatic cancer model in C57BL/6 mice and the second, a spontaneous breast cancer model in transgenic mice carrying the activated rat c-neu oncogene [48]. CD34-negative MSCs were isolated from mouse bone marrow and genetically modified to express the suicide gene HSV-TK under control of the Tie2 promoter/enhancer. Tumor angiogenesis was selectively targeted by making use of the ability of MSCs to act as progenitors for tumor vessel growth and then to activate the HSV TK gene in the context of this event. Expression of the Tie2 gene is largely restricted to angiogenic "hot spots" in tumors. Adoptively transferred MSCs that were recruited to the vasculature of spontaneous breast tumor, or orthotopic pancreatic tumor, induced transgene expression only when the MSC developed endothelial-like characteristics. In both tumor models, locally expressed HSV-TK gene product in combination with ganciclovir treatment resulted in a significant reduction in primary tumor growth, as well as prolongation of life.

MSCs can also function as progenitor cells for Tumor Associated Fibroblasts (TAFs) [48]. TAFs are a key cell type in the establishment and progression of solid tumors. The cytokine CCL5 is induced by recruited MSCs as they encounter tumor in vivo and begin to develop into TAFs. In a second approach, MSC were engineered to express the HSV-TK under control of the CCL5 promoter. The engineered cells were injected into the peripheral circulation of mice with growing orthotopic pancreatic tumors. The effect on tumor growth and tumor metastases was then evaluated. The homing and activation of CCL5 promoter engineered MSC was verified by reporter gene expression. In the presence of ganciclovir, CCL5-HSV-TK engineered MSCs led not only to a significant reduction in the growth of primary pancreatic tumors, but also dramatically reduced the incidence of metastases [49]. A direct comparison of the Tie2 and CCL5 targeting strategies in an orthotopic model of hepatocellular carcinoma showed that while both approaches suppress tumor growth, the CCL5 strategy was more effective [50]. We believe that based on the unique biology of each tumor type, each tumor entity will be more or less susceptible to a specific targeting approach. Therefore, selection of the most effective MSC/suicide gene combination for the specific tumor entity will be most important.

In each of these experiments, the systemically injected stem cells were found largely within the tumor, but they could also be identified in other tissues including skin, gut and secondary lymphatics. Parallel reporter-gene experiments showed that the engineered stem cells recruited to other tissue niches did not undergo the same program of differentiation and activation, and therefore did not express the transgene. Thus, the enhanced targeting of tumor environments using the RANTES/CCL5 or Tie2 promoter largely eliminated the non-specific background expression of the transgene in other organ systems with the exception of the spleen. The spleen has as the most important organ of the reticuloendothelial system has a high clearance capacity for systemically administered cells in general.

Clinical Applications

The approach of suicide gene therapy for cancer has been validated by in vitro and in vivo experiments and in a series of pre-clinical studies. On this basis a small number of clinical trials have been undertaken in humans [51]. As discussed below, there are understandable concerns associated with the administration of genetic material to humans. Therefore the cancers targeted in these early trials of suicide gene therapy against cancer are generally highly aggressive and have limited therapeutic options available.

Glioblastoma Multiforme

Glioblastoma multiforme is the most common and most aggressive form of primary brain tumor in adults [52] with median survival of less than 15 months. In a prospective trial, eight patients with recurrent glioblastoma multiforme were treated with a HSV-TK gene-bearing liposomal vector followed by systemic application of ganciclovir. This therapy resulted in a greater than 50% reduction in tumor volume in two out of eight patients, and focal treatment effects in the remaining patients [12]. This treatment was tolerated without major side effects, supporting the feasibility of suicide gene therapy against glioblastoma multiforme.

Mesothelioma

Mesothelioma is a universally fatal cancer of the pleura. The median survival from presentation of the disease is less than one year [53]. In a phase I clinical trial, cells from the human PA1STK cell line and stably transduced with the HSV TK gene were directly infused into malignant pleural effusions. Subsequently ganciclovir was infused intravenously for seven days. This resulted in a measurable induction of Th1 and Th2 cytokines levels in serum and pleural fluid. However, serial computed tomography did not show demonstrable improvement in the tumor burden [54].

Gastrointestinal Cancer

Hepatocellular carcinoma is the sixth most prevalent cancer, and the third most frequent cause of cancer-related death [55]. Eighteen patients with primary hepatocellular carcinoma one to five cm in diameter or resectable hepatic metastases from colorectal cancer were treated with an adenoviral vector carrying the nitroreductase suicide gene by ultrasound guided injection prior to undergoing resection. Patients were treated with escalating doses of the construct (range, $10(8)$-$5 \times 10(11)$ virus particles). Vector administration was well tolerated with minimal side effects, had a short half-life in the circulation, and stimulated a robust antibody response on ELISA assays. Dose-related increases in tumoral nitroreductase expression measured by immunohistochemical analysis of resected tumors were observed [56]. Tumor size as an outcome parameter was not assessed.

Prostate Cancer

Prostate cancer is the second leading cause of death in men in the United States with death rates exceeding 25,000 per annum [57]. In a human trial sixteen patients who presented with local recurrence of prostate cancer after definitive radiation treatment were treated with replication-competent adenovirus-mediated suicide gene therapy using the CD and HSV-TK suicide genes, along with 5-FC+GCV prodrug therapy. Seven (44%) patients demonstrated a greater than 25% decrease in serum prostate-specific antigen, and three (19%) patients demonstrated a greater than 50% decrease in serum prostate-specific antigen [9]. In a second clinical trial, the nitroreductase system was also used alone for treatment of prostate cancer. Patients with local prostate cancer who were scheduled for radical prostatectomy received direct intraprostatic injections of an adenoviral vector carrying the nitroreductase. This confirmed safety and tolerability of the approach. A second group of patients who had biopsy-proven local recurrence following primary treatment were similarly injected the adenoviral vector carrying nitroreductase. These patients subsequently received systemic infusion of the prodrug CB1954. Treatment was again well tolerated and treated patients had a delay in progression of the tumor marker PSA at 6 months compared to a control group [11]. Further clinical trials have also confirmed the proof of concept of suicide gene therapy of prostate cancer [10]

Gynecologic Cancers

Endometrial cancer is the most common cancer of the female reproductive system with a worldwide incidence of nearly 200,000 cases diagnosed each year [58]. Ovarian cancer occurs in approximately 25,000 new cases, and is responsible for over 15,000 deaths, per annum in the United States [59]. Both cancers have been treated using suicide gene therapy. In a clinical trial of twelve patients, nine patients with ovarian cancer and 3 patients with endometrial cancer were administered infectivity-enhanced adenovirus expressing the HSV-TK suicide gene. Clinical efficacy was determined by comparing pretreatment CT findings with CT findings on day 29 following ganciclovir administration. This revealed stable disease in five patients and progressive disease in seven patients.

Limiting Factors

Suicide gene therapy of cancer shows considerable therapeutic potential. However, routine clinical application in humans is currently limited by concerns over the feasibility and safety of this strategy. One challenge has been suboptimal efficiency in introducing suicide genes into cancer cells. It has proven difficult to achieve introduction of the suicide gene in all cancer cells. There are a number of reasons for this. Viral vectors are targeted by the adaptive and innate immune response. Further, due to the clonal nature of cancer, even a small number of surviving cells can result in disease progression or recurrence.

A second challenge is how to introduce suicide genes into cancer cells over normal non-cancer cells with a high degree of specificity to minimize undesired side effects. Once this challenge is overcome, there remains the concern that local activation of prodrugs may still result in regional and possibly systemic side effects on the host.

Complications

The safety of suicide gene therapy for cancer remains a significant concern. With any therapy involving genetic manipulations, the introduction of suicide genes into target cells has the potential of being tumorigenic in and of itself. For instance, insertion of the suicide gene

may result in unintended dominant gain-of-function mutations that activate oncogenes or loss of a tumor suppressor gene. The potential mechanisms for this include disruption of enhancer or promoter elements in the vector or aberrant splicing of the vector transcript. This became drastically evident in a clinical trial of gene therapy for x-linked severe combined immunodeficiency, in which 2 out of 10 patients developed leukemia due to retroviral integration in proximity to the LMO2 proto-oncogene promotor [27]. Furthermore, pre-clinical trials are frequently undertaken in animal models with relatively short follow-up periods. Therefore the lifetime risk of mutagenesis in a human is difficult to estimate [47].

The second problem occurs from the host immune response to the vector and suicide gene product. For example, an adenovirus based gene therapy trial of ornithine transcarbamylase deficiency resulted in the death of a study patient due to anaphylactic shock in response to the adenovirus vector [24].

Conclusion

Suicide gene therapy is a promising approach to achieving highly specific cancer therapies while minimizing toxic effects of chemotherapy drugs on non-targeted/non-cancerous tissues. A number of suicide gene therapy systems have been developed including HSV-TK / GCV and CD/5-FU. Furthermore there are vectors that allow specific delivery of suicide genes to the cancer environment. These include mesenchymal stem cells, viral vectors and free DNA. On this basis, a large body of evidence supports this strategy in pre-clinical models. However, the therapeutic approach in humans is limited by low efficiency of delivering the suicide genes and concerns regarding safety. Thus the number of clinical trials in human patients has been limited and treatment benefits observed modest. An intensive research effort is necessary to overcome these obstacles and turn suicide gene therapy into real benefit for our patients.

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