

MINIREVIEW

Recent Developments in the Virus Therapy of Cancer (44470)

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Abstract. Cancer is one of the leading causes of death in the United States. Although there has been significant progress in the areas of cancer etiology, diagnostic techniques, and cancer prevention, adequate therapeutic approaches for many cancers have lagged behind. One promising line of investigation is the virus therapy of cancer. This approach entails the use of viruses, such as retroviruses, adenovirus, and vaccinia virus, to modify tumor cells so that they become more susceptible to being killed by the host immune response, chemotherapeutic agents, or programmed cell death. This review discusses recent advances in the virus therapy of cancer from both basic science and clinical perspectives. Given the potential of viruses to kill tumor cells directly or transduce desired gene products to allow a vigorous host antitumor immune response, the virus therapy of cancer holds great promise in the treatment of cancer. [P.S.E.B.M. 2000, Vol 223]

The purpose of this article is to update the reader on some of the recent developments in the virus therapy of cancer. It is not intended to be an exhaustive review of the literature. Several reviews on the subject of virus therapy of cancer and gene therapy of cancer have been published that provide a good background on the subject (1–5). However, due to the rapidity with which this field is developing and changing, there exists a need for a review on the current state of the science.

Host Immune Responses to Cancer Cells

The human immune system has a wonderfully vast array of strategies to identify and reject foreign material from

the body. It is currently thought that some neoplastic cells can trigger immune effector cells to eliminate the tumor or halt its progress. Some of the cell types thought to be important in antitumor immunity are described below.

Macrophages are phagocytic cells that play an important role in the generation of immune responses by presenting foreign antigen to helper T lymphocytes. In addition, macrophages are known to have tumoricidal properties and can kill tumor cells using tumor-necrosis factor (6) or reactive nitrogen intermediates (7). Macrophages have been shown to be cytolytic for colon carcinoma cells (8), ovarian cancer cells (9), and other tumors; however, it is clear by their presence in human tumors that they are insufficient to prevent progressive tumor growth (10).

Natural killer (NK) cells are a unique population of lymphocytes that can kill some tumors in a major histocompatibility unrestricted fashion and without the requirement of prior sensitization (11). The precise *in vivo* role of NK cells in tumor eradication remains to be established, but the data suggest an important function in killing certain types of cancer cells and possibly limiting the metastatic spread of tumors (12, 13). Lymphokine-activated killer (LAK) cells are predominantly interleukin-2-activated NK cells that are

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endowed with the ability to eradicate NK-resistant tumor cells (14). However, the physiologic function of LAK cells and their role in killing cancer cells still needs to be determined. A tumor immunosurveillance function for NK and LAK cells has been stipulated, but has yet to be proven.

T cell-mediated antitumor immunity involves CD8-positive cytotoxic T lymphocytes. In animal models, cytotoxic T cells have been shown to kill chemical- and virus-induced tumors. In humans, lymphocytes called tumor-infiltrating lymphocytes, isolated from a patient's tumor, are capable of killing some types of tumors (15, 16). Composed of T lymphocytes and NK cells, some antitumor cytotoxic T lymphocyte activity can be detected in this population of cells. As an example, cytotoxic T-lymphocyte clones specific for melanoma antigens have been isolated from melanoma-derived tumor infiltrating lymphocytes (17).

With greater than one million new cases of cancer in the United States occurring annually, it is apparent that host immune responses to cancer can be inadequate. We have learned a great deal about how tumors escape or modify the host antitumor immune response.

Tumor-Associated Escape Mechanisms from Immune-Mediated Destruction

There are two major reasons why tumors do not induce a vigorous immune response. First, the tumor can fail to provide a proper antigen for the immune response to detect and to which the immune system can react. Second, the tumor can prevent an immune response by failing to provide proper accessory molecules essential for developing an immune response.

Lack of appropriate antigen presentation can include expressing a mutant tumor protein that is not immunogenic (that is, not capable of eliciting an immune response) (18), having a defective antigen processing pathway so that the antigen cannot be shuttled to the cell surface (19), or masking the tumor antigen so that it cannot be seen by cells of the immune system (20). In addition, release of antigen from the tumor cell surface known as antigenic shedding (21) or loss of antigen through endocytosis-mediated internalization of antigen can occur.

Without the tumor expression of essential surface molecules, no antitumor response can be generated. Absence or decreased expression of class I major histocompatibility molecules prevents the activation of antitumor cytotoxic T lymphocytes (22). Costimulatory proteins, needed for a vigorous host immune response, may be missing from the tumor cell surface. Other escape mechanisms that tumors may use involve resistance to the cytotoxic T lymphocyte lytic molecules and release of tumor-derived immunosuppressive substances such as transforming growth factor- β (23).

Given the complexities of tumor escape from host immune responses, researchers have been investigating ways to circumvent these escape mechanisms. Among other approaches, virus-based therapies are gaining momentum in

the fight against cancer. Viruses can be used in many ways to prevent tumor growth. They can be used to lyse the tumor cells directly, as vectors to transfer genes for immune-enhancing cytokine, or as costimulatory molecules to the tumor cells or to express immunogenic viral proteins in the surface of the tumor cells to evoke a strong host antitumor response. This review will explore two approaches to the virus therapy of cancer. The first approach is the modification of the tumor cells to enhance a host immune response. The second approach seeks to bolster the host antitumor reactivity by manipulating the immune system cells. We will begin by discussing the diverse virus systems currently in use.

Viral Vector Systems

Retrovirus. Retroviruses are single-stranded RNA viruses that contain a viral envelope and encode reverse transcriptase (an RNA-dependent DNA polymerase). Many cancer therapeutic strategies currently being investigated use retroviral vectors. One of the most frequently used retroviruses is the Moloney murine leukemia virus (24–26). Retroviruses preferentially infect dividing cells, thereby targeting actively replicating tumors cells while sparing non-dividing host cells. Through the process of pseudotyping, the host range of retroviruses can be broadened to include cell types the viruses would not normally infect by replacing the normally encoded viral adhesion protein with a protein that would bind to the desired type of host cell. A recent example of this used Moloney murine leukemia virus with a modified envelope protein that bound to human epidermal growth factor receptor that is sometimes overexpressed in human breast cancer cells (25). In addition, a retrovirus was pseudotyped to direct its binding to human hepatoma cells, opening up the potential for liver cancer-directed gene therapy (27). However, retroviruses are not without their disadvantages, which include an approximate 10-kilobase limit on the insertion of nonviral sequences, difficulty producing relatively large amounts of virus, and lack of infection of nondividing tumor cells. As we will see below, despite these difficulties, retroviruses remain an important tool in the virus therapy of cancer.

Adenovirus. Adenoviruses are double-stranded DNA-containing viruses. Adenoviral vectors currently in use have been modified by the removal of the E1A region that controls virus replication. Therefore, virus replication would be prevented in a treated patient. Because the viral vector is replication-incompetent, a specially engineered human embryonic kidney cell line that provides the missing elements for virus replication is required for virus production (28). Adenoviruses have certain advantages over retroviruses that can be exploited. High titers of virus can be produced, and host cells can be infected efficiently by the virus. Of therapeutic importance, adenoviruses infect non-dividing cells and therefore can introduce their DNA into tumor cells that are not actively replicating (29). Numerous

human tumor cell types, which have been transduced using adenovirus vectors, include breast cancer (30), lung cancer (31, 32), prostate cancer (33), and ovarian cancer (34). In spite of the obvious advantages of using adenovirus vectors in the therapy of cancer, significant problems still exist. Host immune response to adenovirus (35, 36) may hamper treatment of previous recipients of adenovirus with the same serotype of adenovirus by neutralizing the virus and therefore preventing expression of the desired gene product. Newer adenovirus vectors seek to make them less immunogenic and therefore less likely to produce neutralizing antibody or a host cell-mediate immune response. Additionally, active investigation is underway to increase the length of expression of the adenovirus-transduced gene (37).

Other Viral Vectors. Adeno-associated virus is a single-stranded DNA virus capable of infecting dividing and nondividing cell types. Adeno-associated virus vectors are being tested in Phase I clinical trials for treatment of cancer and cystic fibrosis (29). Problems include difficulty in producing high virus titers and loss of ability to integrate into the host genome in the current crop of viral vectors. Adeno-associated virus has been used as a vector to transduce several types of tumors including leukemia (38), malignant gliomas (39), and hepatocellular carcinoma (40).

Herpes simplex virus is a large, double-stranded DNA virus that is capable of infecting neuronal tissue. Accordingly, this virus is being studied as a vector for gene therapy of neurons (41, 42). This virus may ultimately be used in the treatment of tumors of neuronal origin. Since herpes simplex virus can establish latency in nondividing neuronal cells, long-term expression of genes can be accomplished (43). A departure from the approach of using herpes simplex virus as a whole virus vector has been to use a single gene from the virus in cancer therapy. Herpes simplex virus thymidine kinase gene expression has been used in a variety of tumor therapy systems as a "suicide gene" strategy. This approach is discussed in more detail below.

Vaccinia virus is another large double-stranded DNA virus that has been used as a viral vector to carry large genetic inserts, up to 25 kilobase pairs of DNA. Vaccinia virus has been used in mouse cancer models to transduce immunomodulating cytokine genes into tumors (44) to enhance the host immune response to the tumor (45).

Modification of Tumor Cells.

Viral Oncolysates. Tumor cells isolated from human cancer patients are notoriously poor inducers of immune responses. Because of this phenomenon, investigators are actively searching for techniques to enhance the immunogenicity of tumor cells. For decades, virus-modified tumor cell membranes, or viral oncolysates, have been known to induce antitumor immunity against nonvirus-modified tumor cells (46–48). The mechanism whereby virus infection of tumor cells induces immunity to noninfected tumor cells is unknown, but virus xenogenization of tumor cells (49), as it is called, has been tried against various tumors using

several different viruses. Oncolysates of influenza virus-infected melanoma, vulvar carcinoma, and ovarian carcinoma have been tested in patients. Newcastle disease virus-infected colon carcinoma oncolysates have been used to treat patients for micrometastases after primary tumor removal (50, 51). Vaccinia virus oncolysates have been used in several studies against melanoma and metastatic breast, kidney, and colon carcinoma. The above studies have been thoroughly reviewed by Sinkovics and Horvath (5). Some patients with stage III melanoma showed an overall survival advantage when treated with vaccinia melanoma oncolysate in a phase III multi-center trial (52, 53). All of the above studies were able to demonstrate a clinical response in some patients or generation of active immunity against tumor antigens, but it is clear more work needs to be done to increase the patient response rate.

An interesting model of tumor eradication in mice involves using reovirus type 3 (Dearing strain). Williams *et al.* (54) demonstrated that L1210 tumor cells pretreated with reovirus and the chemotherapeutic agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and then injected into syngeneic mice were rejected. Subsequently, our studies and those of others have shown that reovirus and BCNU can be used to treat L1210- or EL-4-bearing mice to elicit a significant cure rate (55, 56). Challenging cured mice with homologous tumor results in 100% survival, whereas challenge with heterologous tumor results in the death of all animals. In addition, our laboratory has shown that if the mice are immunosuppressed with cyclosporine during the therapy, the efficacy of the therapy is abrogated (57). These results indicate that the therapy generates an antitumor immune response to result in the eradication of the tumor. One study has suggested that tumor necrosis factor may be involved in tumor elimination in this system (58). The investigation of mechanisms involved in the killing of tumor in the reovirus system is currently underway. The reovirus therapy differs from the above viral oncolysate systems in that pretreatment of tumor with virus is not necessary. Coffey *et al.* (59) reported that reovirus requires an activated *ras* proto-oncogene signaling pathway to infect cultured cells. Since *ras* is activated in about one-third of all human tumors, it was reasoned that reovirus might be used in the treatment of cancer by selectively killing tumors while leaving normal host cells unaffected. They showed that reovirus could cause the regression of human glioblastoma tumor cells in an immunodeficient mouse model. In this system, it is likely that reovirus is acting as an oncolytic agent, selectively replicating in cancer cells and ultimately destroying them. Also, there is experimental data suggesting that recombinant adenovirus, deficient in the E1B gene expression, is thought to be oncolytic for hepatocarcinoma cells (60), some lung cancer cell lines (61), and colon cancer cells (62).

T-Cell Costimulatory Molecules. One of the requirements for T lymphocytes to become activated is that they must bind to a specific peptide fragment that is pre-

sented on a cell surface along with major histocompatibility molecules through the T-cell antigen receptor. In addition, T lymphocytes, through the CD28 cell surface protein, must recognize costimulatory molecules such as the B7 family of proteins (i.e., B7-1 and B7-2) to become fully activated (63, 64). One major problem with tumor cells is that they rarely express the costimulatory molecules required to elicit a T-cell-mediated immune response. T lymphocytes can be rendered nonfunctional if their T-cell antigen receptor is engaged, but there are no costimulatory molecules to complete the activation process. As stated above, lack of expression of costimulatory molecules is one mechanism whereby tumor may escape immune destruction. To circumvent this problem in tumors, costimulatory molecules can be transduced to promote a vigorous antitumor immune response (Fig. 1). A recombinant vaccinia virus containing the murine B7-1 and B7-2 genes was used to transduce a murine carcinoma. Injection of recombinant virus-infected tumor cells into immunocompetent mice led to the rejection of the tumor (65). Using a modified adenovirus construct, the expression of B7-1 and human interleukin-2 in a murine breast adenocarcinoma model elicited complete tumor regression. Interleukin-2 is required by T cells for proliferation in response to antigen. Expression of B7-1 alone resulted in 38% of the animals rejecting the tumor (66). Yang *et al.* (67) used a retroviral vector to transduce the B7-1 gene into human melanoma cells and demonstrated that cytotoxic T lymphocytes could be generated *in vitro* that were specific for the tumor cells. These data support the use of this approach in the study of treatments for other human cancers. It is important to remember that the costimulatory molecules are not the targeted rejection antigens on the

tumor, but rather the molecules aid in generating a vigorous T-cell response to tumor antigens. As elucidated by Roth and Cristiano (1), a potential problem with this approach is the heterogeneity with which some tumor cells might express the costimulatory molecules. In addition, some tumor cells could unpredictably lose the expression of the transduced gene.

Cytokines. Cytokines are hormone-like molecules that play a role in immunoregulation. Some cytokines stimulate, whereas others suppress, an immune response. Because many tumors fail to stimulate an adequate immune response, researchers are studying methodology to enhance immune responses by increasing the production of immunostimulating cytokines. In early cancer therapy, systemic administration of a therapeutic dosage of cytokines produced deleterious effects. For example, when systemic interleukin-2 was given to cancer patients, serious side effects such as fever, chills, tachycardia, and capillary leak syndrome were observed (68, 69). A relatively new approach entails the transduction of cytokine genes into the patient's primary tumor cells. The principal behind this approach is that the very cells that a vigorous immune response is desired against produce the immunostimulating cytokine or combination of cytokines. This provides the advantage of avoiding the adverse systemic effects by producing cytokines locally, while generating the desired antitumor immune response. It is hoped that systemic immunity to tumors can be produced in this manner so that tumor metastases might be eradicated. Recent preclinical studies (i.e., animal models) have demonstrated the feasibility of transducing tumor cells with cytokine genes. Primary and metastatic murine hepatocellular carcinoma was treated with an interleukin-2-expressing adenovirus vector (70). The investigators found that approximately half of the mice exhibiting primary or metastatic cancer that were treated with the vector went into long-term remission. An accumulation of macrophages and T lymphocytes was noted in the survivors, suggesting the generation of an antitumor response. In another study, an adenoviral vector was used to transduce the human interleukin-2 gene into freshly isolated human lung adenocarcinoma cells (71). Treatment of pre-existing tumor in a mouse model with the gene-modified tumor cells cured those animals, but subcutaneous tumor was not eliminated. Dong *et al.* transduced an interferon- β gene, using a retroviral vector, into murine UV-2237m fibrosarcoma cells and showed an inhibition of tumor growth (72). A growth inhibitory effect of interferon- β on tumor growth along with enhanced natural killer cell cytotoxicity may have accounted for this effect. Viral vectors have been used to transduce cytokines such as interleukin-4 (73, 74), interleukin-7 (75), interleukin-12 (76-78), granulocyte macrophage-colony stimulating factor (79), and a nonsecreted form of tumor necrosis factor- α (80, 81) into tumors of diverse types with each cytokine having a decidedly negative impact upon tumor growth.

Pitfalls of transducing cytokine genes into patient tu-

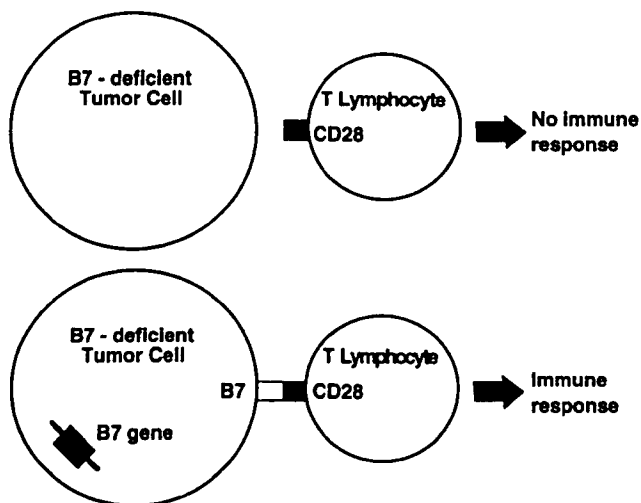


Figure 1. Cytotoxic T lymphocytes must recognize costimulatory proteins, such as B7, on the surface of tumor cells to become activated and express their cytolytic function. However, as an immune escape mechanism, many tumor cells lack proper expression of costimulatory molecules and thus escape an immune response. One potential virus therapy of cancer seeks to restore the expression of costimulatory molecules by transducing the appropriate gene into the tumor cells, thus enabling the tumor cells to properly activate cytotoxic T lymphocytes.

mor cells *in vitro* include the lack of adequate quantity and quality of patient tumor cells and the heterogeneous expression of the cytokine genes. In addition, the tumor cells must be irradiated prior to reintroduction into the patient, and in some instances this adversely affects the production of cytokines. It is likely that different cytokines will be needed to promote antitumor immunity to the different types of cancer. Given that most of the research using cytokine gene-transduced tumor cells has been in animal models, it remains to be proven whether the incorporation of cytokine genes into tumor cells works satisfactorily in a clinical setting.

Drug Sensitivity. One of the disadvantages of traditional chemotherapy is that it usually destroys a significant number of normal host cells. This effect can lead to an unacceptable amount of patient morbidity and mortality. In an effort to localize the effects of chemotherapy to the tumor, enzyme genes can be transduced to the tumor whose products can convert relatively low toxic prodrugs into powerful tumor-killing substances within the tumor itself. Since not all of the patient's tumor cells can be transduced with the gene, a bystander effect may promote the destruction of tumor cells not transduced with the gene (Fig. 2). With respect to the bystander effect, gap junctional intercellular communication was found to be important in the mediation of the outcome. In cell cultures that lack intercellular communication, the bystander effect was absent (82). However, other factors may be important in promoting the bystander effect and may include hemorrhagic tumor necrosis that induces an influx of lymphoid cells to attack

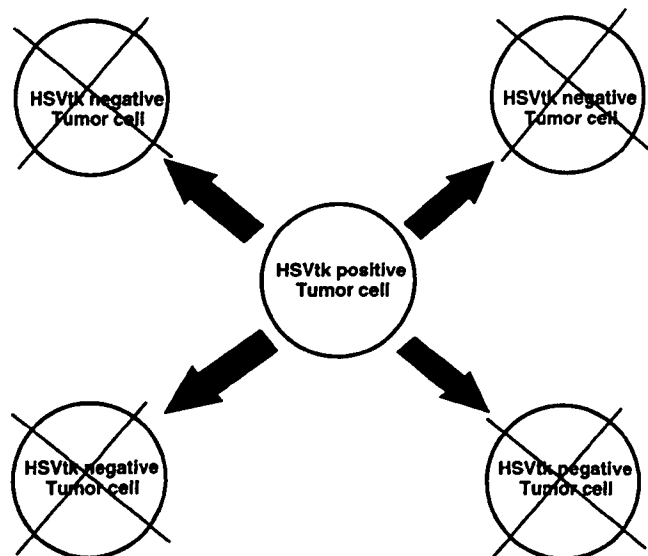


Figure 2. One "suicide gene" strategy to kill tumor cells uses the transduction of the herpes simplex virus thymidine kinase gene into a population of tumor cells. Upon the addition of gancyclovir, toxic metabolites are generated to destroy the tumor. However, since not all tumor cells can be transduced effectively with the herpes simplex virus thymidine kinase gene, effective tumor eradication bystander killing of nontransduced tumor cells must occur. Factors important to bystander killing include intercellular communication, hemorrhagic tumor necrosis of transduced tumor cells, and apoptosis induced by the uptake of the remains of dead tumor cells.

remaining tumor cells. Also, uptake of the remains of dead tumor cells may induce apoptosis of live tumor cells in the same locality.

Examples of these "suicide gene" strategies include herpes simplex virus thymidine kinase (HSVtk)/gancyclovir, *E. coli* cytosine deaminase/5-fluorocytosine, *E. coli* nitroreductase/5-(aziridin-1-yl)-2,4-dinitrobenzamide systems (Table I). Adenoviral and retroviral vectors are commonly employed to transduce the tumor cells with the desired gene. The HSVtk gene was transduced using an adenovirus vector to successfully render human ovarian epithelial cancer cell lines sensitive to killing by gancyclovir (83), and a potent bystander effect was noted. Gancyclovir is converted to a triphosphate form and competes with normal nucleotides to result in DNA chain termination and subsequent cell death. Phase I clinical trials using malignant mesothelioma patients that were administered an adenovirus vector-base HSVtk system demonstrated few side-effects and documented gene transfer to tumor in just over half of the patients (84). The HSVtk gene was under the control of a Rous sarcoma virus promoter and enhancer, and the replication-incompetent virus was administered *via* intrapleural injection. Phase I clinical trials, using prostate cancer patients given adenovirus-HSVtk directly into the prostate followed by gancyclovir intravenously, demonstrated that this regimen could be administered safely (85). Objective responses were noted in three patients, documented as a fall in serum prostate-specific antigen, suggesting anticancer activity of the gene therapy. Recurrent glioblastoma was shown to be targeted effectively by a retroviral vector carrying the HSVtk gene in phase I/II trials (86). The therapy regimen was well tolerated by the patients, and significant therapeutic responses were noted. Colorectal, pancreatic, and thyroid cancer cells lines were retrovirally transduced with the *E. coli* nitroreductase gene and exposed to the prodrug 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) (87, 88). This prodrug is a weak alkylating agent that is converted by the *E. coli* enzyme to a powerful alkylating agent that crosslinks DNA. Significant cell killing was achieved with all tumor types, and relatively strong bystander effects were noted. There are potential problems with the transduced drug sensitivity approach. Severe hepatotoxicity was seen in a rat tumor model that used an adenovirus vector carrying the HSVtk gene (89). These data illustrate that some nonmitotic tissues can take up and be harmed by the suicide gene. Presumably, monitoring of liver function in patients will be essential when using this vector. In addition, some tumors might not be infected readily by the viral vector used or might give a suboptimal bystander effect as some investigators found in a medulloblastoma tumor model (90).

Antisense Inhibition of Tumor Cell Multiplication. Although the development of cancer requires the accumulation of mutations in a number of genes, including oncogenes and anti-oncogenes, one potential strategy to shut down cancer cell proliferation would be to administer

Table I. Virus Etiology of Certain Cancers

Family	Type	Associated cancer
Human papillomavirus	HPV-16 and HPV-18	Cervical cancer
Herpesvirus	Epstein-Barr virus	Burkitt's lymphoma Nasopharyngeal Carcinoma
Retroviruses	Human T-lymphotropic virus I Human T-lymphotropic virus II	Adult T-cell leukemia Hairy cell leukemia
Hepadnavirus	Hepatitis B virus	Liver cancer
Flaviviridae	Hepatitis C virus	Liver cancer

antisense oligonucleotides that could bind to oncogene mRNA. Several mechanisms have been proposed to explain the inhibitory effect of antisense oligonucleotides including binding of sense and antisense strands by Watson-Crick base-pairing with the consequent reduction in transcription, splicing, and translation of the mRNA (91). The effect would be to remove the driving force behind the unrelenting cell replication, characteristic of cancer cells. Steiner *et al.* (92) demonstrated that transducing an antisense *c-myc* gene into prostate tumor cells by using a retroviral vector resulted in a significant shrinking of tumor size compared to controls when the tumor was grown in immunodeficient mice. Using an antisense *c-fos* retroviral vector, one laboratory was able to demonstrate a growth-inhibiting effect on human breast cancer cells grown in immunodeficient mice (93). A lack of host toxicity was noted in treated animals. Tumor cell differentiation was induced in *ex vivo* transduced tumor cells. In addition, the growth of a human lung cancer cell line was inhibited in immunodeficient mice when retrovirally transduced with *K-ras* antisense RNA (94, 95). *K-ras* expression and cell line proliferation were severely reduced.

Viral transduction of antisense genes into tumor cells extends the longevity of the antisense molecules within the cell compared to other techniques used to get antisense molecules inside the tumor cells. Clearly, further investigation into the mechanisms of action of antisense inhibition of tumor cell growth needs to be performed. In addition, improvement needs to be made in the absolute number and the percentage of tumor cells that can be transduced as a result of *in vivo* administration of antisense-containing virus.

Tumor Suppressor Genes. Mutation of tumor suppressor genes plays an important role in cancer development. Approximately half of all tumors have functional impairment of the *p53* gene product. One therapeutic strategy is to use viral vectors to transduce a functional copy of the *p53* gene into tumor cells. This can result in arrest of tumor cell growth and induction of apoptosis (96, 97). A recombinant vaccinia virus expressing wild-type *p53* produced growth inhibition and induced apoptosis in human and rat glioma tumor cell lines *in vitro* (98). An adenovirus vector expressing wild-type *p53* was able to induce apoptosis in human pancreatic cancer cells *in vitro* and in an immunodeficient mouse model that used subcutaneous tumor (99). Chemotherapy-resistant human breast cancer cells were rendered susceptible to chemotherapy following treat-

ment with a recombinant adenovirus vector expressing wild-type *p53* (100). A similar vector was used to kill malignant human glioma cells in an immunodeficient mouse model (101). Two recent phase I clinical trials used adenovirus-mediated wild-type *p53* gene transfer to patients with advanced non-small-cell lung cancer (102, 103). Both studies demonstrated that the therapy was well tolerated by the patients, produced expression of the wild-type *p53* protein, and gave evidence of antitumor activity in some of the patients. These encouraging studies will surely prompt researchers to investigate whether other tumor suppressor genes can be used to slow or halt tumor growth.

Modification of Host Cells

Modified Tumor-Infiltrating Lymphocytes. Tumor-infiltrating lymphocytes (TIL) are generally T lymphocytes that are specific for the tumor from which they were isolated (104, 105). TIL are major histocompatibility complex class I-restricted and presumably recognize tumor antigens being presented on class I-expressing tumor cells. Though some success has been achieved by treating melanoma patients with autologous TIL that were expanded *in vitro* prior to reinfusion, there is a need to improve the response rate and the duration of response. Two approaches have been employed recently to improve the effectiveness of TIL. The techniques involve the transduction of cytokine genes that enhance the activity of the TIL or chimeric receptors that redirect the nonspecific TIL to lyse the desired tumor cells (106). Tumor-necrosis-factor (TNF) retrovirally transduced human TIL were shown to produce large quantities of TNF relative to nontransduced control TIL (107). In another study, tumor necrosis factor-transduced TIL were capable of increased killing of autologous pancreatic tumor cells compared to controls (108). Current investigations are centering on improving viral vectors to boost cytokine production in TIL. Using retrovirally transduced chimeric receptor genes, Hwu *et al.* (107) altered the specificity of TIL to recognize a human ovarian tumor cell line. An antibody variable region specific for ovarian tumor conferred specificity on the TIL. The data show that TIL were able to recognize and lyse specifically the ovarian tumor cell line. Another laboratory redirected the specificity of cytotoxic T lymphocytes to recognize and lyse a renal cell carcinoma line (109). One of the biggest obstacles that still needs to be

Table II. Examples of "Suicide Gene" Strategies

Enzyme	Source	Prodrug	Cytotoxic action
Thymidine kinase	Herpes simplex virus	Gancyclovir	Inhibits DNA synthesis
Cytosine deaminase	<i>E. coli</i>	5-Fluorocytosine	Inhibits DNA synthesis
Nitroreductase	<i>E. coli</i>	CB 1954	DNA intrastrand crosslinking

overcome using modified TIL includes improving TIL capacity to traffic back to the patient's tumors (110).

Although not classified as TIL, natural killer cells are a population of lymphocytes that can kill some tumors in a major histocompatibility unrestricted fashion and without the requirement of prior sensitization (11). Retroviral transduction of human natural killer cell lines with the *interleukin-2* gene resulted in stable expression of the cytokine and enhanced tumoricidal activity against tumor cell lines (111).

Drug Resistance of Normal Host Cells. Since many chemotherapeutic agents act indiscriminantly on replicating cells, not only are multiplying tumor cells destroyed, but also host tissue that is proliferating is also at risk. Patient bone marrow myelosuppression is a particular problem, and the speed at which the bone marrow recovers frequently dictates how quickly the next course of chemotherapy can be given to the patient. In an effort to minimize the destruction of bone marrow precursor cells, the multiple drug resistance gene (*MDR1*) has been transduced into CD34⁺ stem cell-enriched populations (112, 113). The *MDR1* gene product, also known as P-glycoprotein, acts by providing resistance to naturally derived lipophilic chemotherapeutic agents by pumping these agents out of the cell. Examples of the drugs to which P-glycoprotein can confer resistance include the anthracyclines, vinca alkaloids, and actinomycin D. The purpose of conferring drug resistance to normal host cells is two-fold. Clearly, reduction of bone marrow myelosuppression is paramount, but it also may be possible to use higher doses of chemotherapy against the patient's tumor if bone marrow precursors express the *MDR1* gene product, thus potentially eliciting greater damage to the cancer cells.

Recent studies have shown that human hematopoietic cells can be transduced with the *MDR1* gene using an SV40 pseudoviral vector (114). Gene transfer was noted to be efficient, and significant P-glycoprotein expression was shown. In a clinical study, two of five patients whose cells were transduced with an MDR cDNA-containing retrovirus showed transduction of bone marrow cells at 10 weeks post-transplantation (115). The investigators postulated that the MDR-transduced cells might not compete as well as the co-infused non-MDR-transduced cells during the repopulation of the patient's bone marrow, accounting for the three patients that showed negative results. In addition to low bone marrow repopulation problems, there is always a risk of transducing the *MDR1* gene into patient cancer cells residing in the processed bone marrow samples. Clearly, this

type of approach holds great promise, but several important technical details still need to be worked out.

Viral Vaccines to Prevent Cancer

Although viruses are thought to cause only 10%–15% of all cancers, this still amounts to a very serious threat (116). One way in which viruses can be used to prevent virus-induced cancers is through immunization of the host against a particular virus. If a humoral (i.e., antibody-inducing) or cell-mediated immune response can be generated through viral vaccination, then the process leading to the generation of cancer can be halted. The best illustration of a virus, in which vaccination has been demonstrated to decrease the risk of cancer, is the hepatitis B virus (117). Other known or alleged cancer-inducing viruses in which viral vaccines may reduce cancer risk include human papillomavirus (118), Epstein-Barr virus (119), human immunodeficiency viruses (HIV-1 and HIV-2), human T-lymphotropic viruses (HTLV-1 and HTLV-2), and hepatitis C virus (Table II).

Whereas neutralizing antibody may be important in primary viral infection, established virus infection requires cell-mediated immunity, particularly cytotoxic T lymphocytes. The challenge for vaccine investigators is to understand how to deliver the virus antigen preparations to elicit potent antiviral cell-mediated immunity. Certainly, prevention of virus-induced cancers is desired over having to treat established cancer, and viral vaccines are a promising way in which the number of patients with certain cancers can be diminished. As we learn more about the mechanisms by which viruses can act as cofactors in cell transformation, other viruses may emerge as targets for viral vaccines against cancer.

Summary

Given the recent advances in the development of suitable virus vectors and their application to numerous tumor cell types, the future of virus therapy of cancer holds much promise. The use of viruses in viral oncolysates and as agents to transduce desired gene products opens up another avenue in treating cancer. Much work remains at both the basic science and clinical levels, but preliminary results are encouraging that, as an adjunct to more traditional cancer therapy, or possibly as a stand-alone therapy, the use of viruses in the treatment of cancer has a favorable future.

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