

Viral Vectors for Cancer Gene Therapy: Viral Dissemination and Tumor Targeting

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Abstract: Cancer gene therapy is the most promising and active field in gene therapy treatment. Although previous experimental and clinical trials have brought forward some exciting cases, in general, the clinical benefits have been limited. A major difference between virus-mediated gene therapy and other therapies is the poor physical diffusibility of viral vectors, which is also one of the major obstacles in cancer gene therapy. As safety is a prerequisite to enhanced viral dissemination, tumor-specific targeting becomes crucial. The present review focuses on questions related to efficient viral dissemination in tumor masses and how to sustain a high level of oncolytic virus targeting of tumor cells only. We will first consider two common reasons for limited virus spread in tumor masses and then discuss strategies for improving the tumor-specific oncolysis of currently used viral vectors and to comment on their advantages and potential problems.

INTRODUCTION

Gene therapy was originally designed to treat inherited diseases that appear as a result of defective genes. In actuality, however, most efforts have been applied for correcting genetic abnormalities affecting somatic tissue, primarily cancers. According to statistics of the Journal of Gene Medicine Database, around 63.4% of the total clinical gene therapy trials has involved cancer treatment [Thomas, *et al.*, 2003]. Gene therapy has been frequently used clinically for cancers of breast, lung, colon, prostate, and brain as well as in leukemia. All trials have confirmed the safety of using viral vectors in human cancer treatment. At the present time, most clinical studies involving cancer gene therapy are still in the phase I stage and only a few have progressed to phase II/III trials. The strategies of these vectors for cancer treatment were to deliver potential therapeutic genes including anti-angiogenic factors [Arafat, *et al.*, 2000; Hirschowitz, *et al.*, 2002; Ma, *et al.*, 2002; Matsumoto, *et al.*, 2001; Shi, *et al.*, 2002; St George, 2003; Tanaka, *et al.*, 1997; Tanaka, *et al.*, 1998], tumor-suppressor or apoptotic genes [Cheney, *et al.*, 1998; Hall, *et al.*, 2000; Hemmati, *et al.*, 2002; Hlavaty, *et al.*, 2000; Huh, *et al.*, 2001; Katner, *et al.*, 2002; Roth, *et al.*, 1998; Shimada, *et al.*, 2002; Steiner, *et al.*, 2000; Timiryasova, *et al.*, 2001; Wilson, 2002; Wu, *et al.*, 2001], prodrug-activating genes [Aghi, *et al.*, 2000; Boviatsis, *et al.*, 1994; Culver, *et al.*, 1992; Ebara, *et al.*, 2002; Jia, *et al.*, 1994; Kubo, *et al.*, 2003; Okabe, *et al.*, 2003; Sutton, *et al.*, 2000; Ueda, *et al.*, 2003; Wierdl, *et al.*, 2001; Zhang, *et al.*, 2003] and immunostimulating genes [Akiyama, *et al.*, 2002; Gomella, *et al.*, 2001; Guan, *et al.*, 2001; Mazzolini, *et al.*, 2003; Park, *et al.*, 2003; Rochlitz, *et al.*, 2003; Ruzek, *et al.*, 2002]. While delivery and expression of therapeutic genes using viral vectors is still an important approach for cancer treatment, there has been no major breakthrough for its clinical

benefit [Rainov, 2000]. Preliminary clinical results of most phase I trials have shown mild benefits in tumor response [Kubo, *et al.*, 2003; Markert, *et al.*, 2000; Merritt, *et al.*, 2001; Nemunaitis, *et al.*, 2001; Post, 2002; Rainov, 2000; Trudel, *et al.*, 2003; Vasey, *et al.*, 2002]. It has been recognized that viral dissemination in tumors is crucial for achieving a satisfactory clinical efficacy and that oncolytic, replication-competent viral vectors might be the most promising for cancer treatment [Bell, *et al.*, 2003].

There are numerous recent review articles covering various oncolytic viral vectors and a number of different tumor-targeting strategies [Bell, *et al.*, 2003; Burton, *et al.*, 2002; Dobbstein, 2003; Fillat, *et al.*, 2003; Lundstrom, 2003; Post, 2002; Rots, *et al.*, 2003; St George, 2003; Thomas, *et al.*, 2003; Whitehouse, 2003]. This review will focus on questions of efficient viral dissemination in tumor masses and how to sustain a high level of oncolytic virus targeting of tumor cells only.

PROBLEMS OF VIRUS DISSEMINATION IN TUMORS

Viral vectors used for cancer gene therapy can be divided into three categories: a) replication-deficient but carrying a potential therapeutic gene; b) replication-competent viruses that lyse tumor cells through lytic replication (oncolytic) and c) oncolytic viruses for use with a therapeutic gene. For categories a) and c), one must decide which therapeutic gene is to be carried, based on knowledge of the gene and the cell biology of the targeted cancer type. In this section, our discussion is focused on difficulties related to virus dissemination in the tumor mass, which is a common problem for all three categories of viral vectors used in cancer gene therapy.

a) Poor Mobility of Viral Particles in the Tumor Mass

Viral vector dissemination within a tumor is significantly hindered by the extremely limited diffusibility of virions within extracellular spaces in these tumor masses. Solid

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tumors are masses with or without outside capsules. Due to high proliferation and loss of contact inhibition, the cell density of the tumor mass is usually much higher than that of normal tissues and those masses often contain a large amount of fiber tissue. Evidence has shown that these supporting structures in a tumor can effectively prevent virus spread [Sauthoff, *et al.*, 2003]. Moreover, viral vectors are large particles with diameters ranging from 50-200 nm. In addition, enveloped or non-enveloped viral particles usually carry substantial electrical charges on their surface, which render them very “sticky” to proteins on extracellular matrices and on the cytoplasmic membrane. Therefore, these particles have an extremely low mobility in a tumor mass. (Fig. 1) shows a diagram based on our observations of glioma animal models intratumorally-infected with recombinant Herpes Simplex Virus type-1 (HSV-1). Using immuno-cytochemistry with a polyclonal antibody against HSV-1 viral proteins, we found that injected virions may infect only 5 layers of the tumor cells surrounding the needle track to form an infection zone, even using an rr⁻ (ribonucleic reductase mutation) HSV-1 virus (hrR3) that can theoretically replicate in tumor cells but not in non-proliferating cells (oncolytic) [Goldstein, *et al.*, 1988].

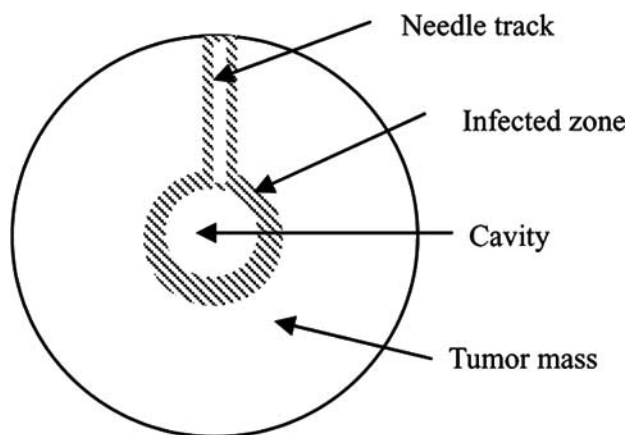


Fig. (1). Diagram of viral dissemination in a tumor mass. The wall of the needle track and the cavity is formed by a few layers of infected tumor cells (infection zone). Due to poor mobility of viral particles, most of the cells in infection zone are infected with a very high MOI, which prevents efficient viral replication and dissemination in the tumor mass.

b) Host Immune Response to Block Viral Dissemination

Another limiting factor for viral dissemination *in vivo* is the host immune response. Theoretically, all viruses are more or less antigenic and host immune responses are inevitable. Some viruses, such as adenovirus, are known for their strong immunity (for recent reviews) [Bennett, 2003; Jooss, *et al.*, 2003; Liu, *et al.*, 2003; Lowenstein, *et al.*, 2003]. Immune responses to virus vectors include both specific adaptive responses and non-specific innate responses [Chen, *et al.*, 2003; Ferrari, *et al.*, 2003; Wakimoto, *et al.*, 2003]. The latter has been found to play an important role in eliminating adenoviral vectors [Liu and Muruve, 2003] and HSV [Wakimoto, *et al.*, 2003]. The

innate response to viruses may be transcriptionally independent, i.e. it is caused by viral envelopes and capsids. In the case of HSV, the innate response is the first line of host defense in both naïve and immunized individuals. The innate responses result in the lysis of virions and virus-infected cells as well as the initiation of mechanisms leading to effective adaptive immunity [Wakimoto, *et al.*, 2003]. In the case of adenovirus, innate responses cause inflammation of transduced tissues and substantial loss of vector genomes within the first 24 h of inoculation [Liu and Muruve, 2003].

c) Short Duration of the Lytic Effect in a Tumor

Even for oncolytic viral vectors whose only function is to destroy tumor cells through viral replication, it has been found clinically in tumor samples that activity levels of viral replication in vector-treated tumors were low and short-lived but that the viral DNA remained in tumor samples longer, indicating inactivation of viral gene expression in the tumor after inoculation [Markert, *et al.*, 2000; Nemunaitis, *et al.*, 2001; Vasey, *et al.*, 2002]. The mechanism of this self-limiting effect in oncolytic virus has not been studied thoroughly.

STRATEGIES FOR TUMOR-SPECIFIC DISSEMINATION OF VIRAL VECTORS

Any good cancer treatment must meet two requirements: efficacy and safety. For virus-mediated gene therapy, efficient intratumoral dissemination is a key precondition for former, but it also represents a major potential risk factor in terms of safety.

The early viral vectors used in clinical trials for cancer were all very attenuated mutants. The majority of these vectors were either replication-deficient or substantially impaired. This may represent a safety concern with respect to using viral vectors during the early development of virus-mediated gene therapy. According to the database of the Journal of Gene Medicine, there have been more than 400 clinical gene therapy trials for cancer treatment and the majority used virus-based vectors. To date, no serious adverse responses have been reported, suggesting the relative safety of the currently used viruses. However, in contrast to small molecules, physical diffusion of virions within a tumor mass is extremely poor, and the spreading of viral particles is almost totally dependent on biological dissemination. Therefore, it has been widely accepted that more aggressive viruses may be necessary to facilitate viral spreading in the tumor.

On the other hand, aggressive virulence must be accompanied with highly specific targeting to ensure the safety for oncolytic viral therapy. Specific targeting for oncolytic viruses is not only necessary from the standpoint of safety but can also potentially enhance the efficacy of the viral vectors. The goal of specific targeting for oncolytic virus can be achieved through different strategies, which are designed according to the biological nature of the virus to be used. As shown in (Fig. 2), the life cycle of lytic virus has many stages. Theoretically, each step in its life cycle can be considered in designing a targeting strategy.

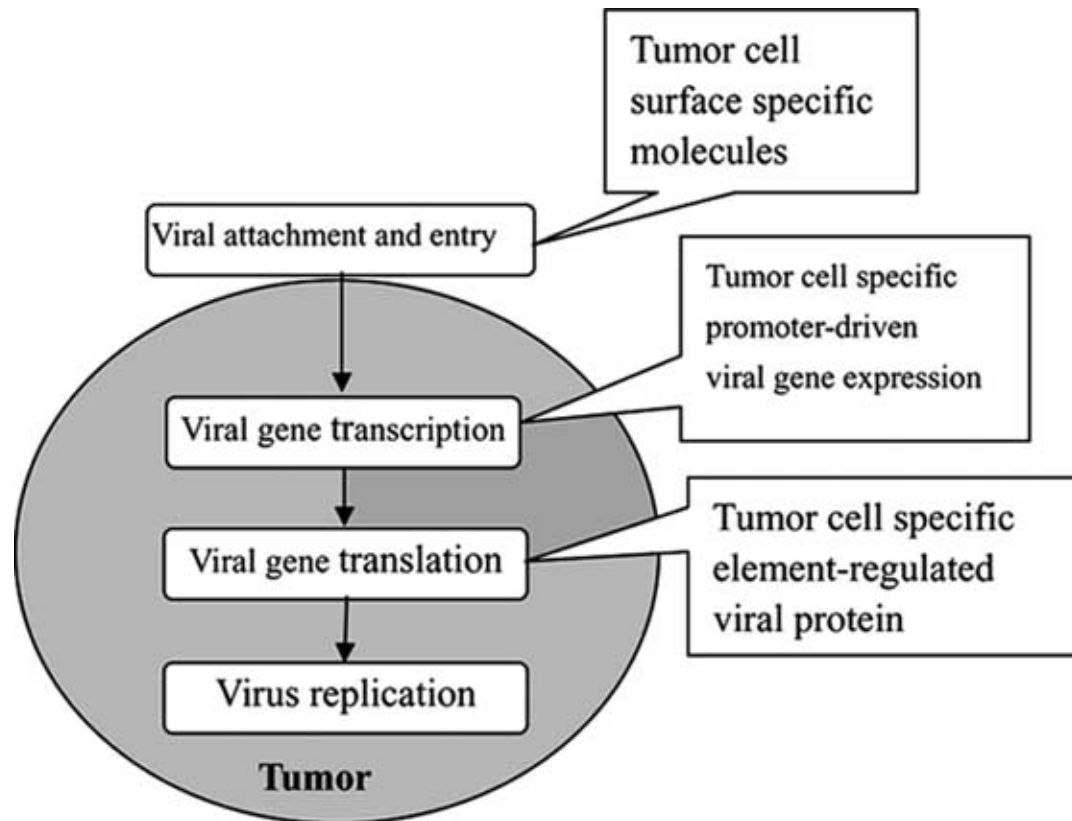


Fig. (2). Strategies of oncolytic viruses for tumor specificity. By targeting each stage of viral replication, recombinant viruses can be created specifically for tumor lysis.

CELL TYPE-SPECIFIC SURFACE MOLECULE TARGETING

This strategy is based on the understanding that viral entry to the host cell requires a specific interaction between the viral protein and cellular receptors. Numerous efforts have been made to change viral surface proteins in such a way as to re-direct its tropism. In the case of adenovirus, since infectivity is limited due to a lack of CAR expression on the surface of many types of cells, it was proposed that small peptide motifs possessing receptor binding specificities could be incorporated into the carboxy terminus of Ad fiber protein, thus enabling the virus to attach and infect *via* a novel cell surface receptor [Michael, *et al.*, 1995]. Wickham *et al.* further supported this concept by incorporating a heparan-binding domain into the C-terminus of the fiber protein. The resulting recombinant virus had a broadened tropism that enabled the virus to bind to widely expressed, heparan-containing cellular receptors and delivered genes to multiple cell types at markedly higher efficiencies than unmodified Ad [Turturro, 2003; Wickham, *et al.*, 1996]. Most recently, Fontana *et al.* reported a novel approach for redirecting the tropism of adenovirus by combining random phage lambda display technologies [Fontana, *et al.*, 2003]. Ad5 fiber knob domain, the portion which interacts with the cellular receptor CAR, was expressed on the capsid of phage lambda and a large collection of ligands in the HI lop of Ad5 knob was constructed. Interestingly, through library panning on the CAR- negative mouse NIH 3T3 fibroblast, three clones of phage lambda were found with increased binding

to these cells. Adenoviruses incorporating these ligands in the fiber gene transduced NIH 3T3 cells 2 or 3 orders of magnitude better than the parent vector. Similar strategies can be employed with many other types of cells to create adenoviruses with a specific tropism to a particular cell type. For tumor-specific binding, some receptors are obvious targets, such as receptors of Epidermal Growth Factor (EGF) or Insulin-like Growth Factor 1 (IGF1) both of which are highly expressed on the surface of cancer cells. This has been successfully demonstrated with measles virus. Measles virus binds to cells through attachment of its protein hemagglutinin (HA) to cellular receptor CD46. Schneider *et al.* showed that measles virus with hemagglutinin fused to EGF or IGF1 (H/EGF and H/IGF1) were able to bind to and replicate in rodent cells expressing the EGF receptor and IGF receptor, respectively [Schneider, *et al.*, 2000]. As CD46 is not present on rodent cells, these results demonstrated that virus binding had been successfully redirected. Indeed, this was the first demonstration that large specificity domains covalently linked to a viral glycoprotein support not only the binding to a new receptor but also efficient cell entry *via* the targeted receptor [Ring, 2002]. Instead of fusing the target ligand to fiber protein, a bifunctional crosslinker has been developed that is an antibody directed against knob chemically conjugated with fibroblast growth factor (FGF) [Rogers, *et al.*, 1997]. Adenovirus treated with this crosslinker showed higher affinity to tumor cells that express high levels of FGF receptors [Curiel, 1999; Douglas, *et al.*, 1999; Kleeff, *et al.*, 2002]. This type of adenovirus is

currently being used in a phase I clinical trial. A similar strategy has been adopted for oncolytic Semliki Forest virus vectors [Lundstrom, 2002].

Another strategy for altering the tropism of viral vectors is pseudotyping, which is achieved by substitution of virus receptor-binding proteins with those from other viruses. The earliest form of pseudotyping was in Moloney murine leukemia virus (MLV), in which the MLV envelope glycoprotein was pseudotyped with the G protein of vesicular stomatitis virus (VSV-G) [Witte, *et al.*, 1977]. This type of modification confers an extremely broad host range and markedly stabilizes the vector particles, allowing vector stocks to be concentrated to high titres. It has been shown that VSV itself can be pseudotyped to bind to specific cell receptors such as those for CD4 and CXCR4 [Schnell, *et al.*, 1996]. Since VSV may replicate more efficiently in transformed cells [Stojdl, *et al.*, 2000; Stojdl, *et al.*, 2000], substituting the viral envelope protein with tumor-specific receptors may further convert this virus to an oncolytic vector for cancer treatment.

While redirecting the tropism of adenovirus by altering viral surface proteins has been quite successful for adenovirus vectors, it may be more difficult with other viruses that have a wide range of hosts. For instance, HSV virus has a number of glycoproteins on its envelope [Rajcani, *et al.*, 1998; Roizman, 1996]. Although gD is the major protein for viral entry, other glycoproteins such as gC and gB also participate in facilitating viral binding [Bender, *et al.*, 2003; Cheshenko, *et al.*, 2002; Mardberg, *et al.*, 2002; Rux, *et al.*, 2002]. The targeting of HSV to a specific cell type not only requires the alteration of gD but also the elimination of the binding characteristics of other glycoproteins, which may affect their functions.

TUMOR-SPECIFIC VIRAL REPLICATION

Modification of viral tropism for tumor-specific binding is one of the best strategies for targeted gene therapy as these viral vectors will potentially have a satisfactory level of bio-distribution throughout the body. This feature is invaluable when one considers that the vectors for destroying metastatic tumors are delivered intravenously. However, it may be that one can always re-direct a virus toward a capacity for tumor-specific binding but it might be impossible to completely block recombinant virus infection of non-tumoral cells. Thus, a second strategy is needed to control undesirable viral replication in host cells.

Generally speaking, all virus growth is favored in actively proliferating cells. However, some viruses are particularly oncotropic by nature. These viruses include human reovirus [Hashiro, *et al.*, 1977], the parvoviruses H-1 [Maxwell, *et al.*, 2002; Rommelaere, *et al.*, 1991] and minute virus of mice [Cornelis, *et al.*, 1990]; VSV [Stojdl, *et al.*, 2000]; and Newcastle disease virus (NDV) [Lorence, *et al.*, 1988]. These naturally occurring oncolytic viruses usually have no or very mild clinical symptoms under normal conditions. Their oncolytic nature appears to result from a tumor-associated deficiency in the interferon response pathway [Stojdl, *et al.*, 2000; Strong, *et al.*, 1998]. In normal cells, reovirus infection causes activation of dsRNA-activated protein kinase (PKR), which phosphorylates the

alpha-subunit of eIF-2, resulting in the termination of translation of viral transcripts [Strong, *et al.*, 1998]. In cells with an upregulated Ras pathway, such as tumor cells, the PKR pathway is impaired, which allows virus replication to proceed. All three oncolytic viruses (NDV, parvoviruses H-1 and reovirus) have been used in phase I clinical trials, and results of the NDV phase I trial have been reported to be quite promising [Pecora, *et al.*, 2002].

HSV was the first virus to be used in demonstrating that deletion in a certain viral gene may render a virus oncolytic [Jia, *et al.*, 1994; Martuza, *et al.*, 1991]. In the case of HSV, a deletion in genes of thymidine kinase(tk), ICP34.5 or ICP6 (ribonucleotide reductase(rr)) all resulted in mutants that could only replicate in proliferating cells. Deletion of tk or rr was found to cause a dependency of viral replication on cellular machinery that is only present while the host cell is in a proliferative state. However, a deletion in the tk gene abolishes the response of the mutant to antiherpetic drugs. Therefore, only mutants with deletions in ICP34.5 and ICP6 genes were tested in clinical trials (referred to earlier).

In recent years, early gene-region deletion mutants of adenovirus have provided another example of conditionally replicating oncolytic virus, although the oncotropic mechanisms of this mutant are more complicated than those of HSV. These mutants include those with deletions in E1A-CR2 or E1B-55kD. The original rationale was that adenovirus proteins of E1A and E1B regions could force the cell to enter S-phase to facilitate viral replication through binding to Rb and P53, respectively. Deletions in these two regions would inhibit viral replication in cells with normal Rb and P53, but they may not affect the efficiency of viral replication in tumor cells since more than 50% of these cells are deficient in Rb and P53. The initial experiments showed that an E1B-55kD deletion mutant dl1520 [Onyx-015] [Cohen, *et al.*, 2001] replicated at the same level as wild-type virus in P53⁻ cells but with a 100-fold lower efficiency than the wild type in P53⁺ cells [Bischoff, *et al.*, 1996]. This finding led to numerous studies on similar adenovirus mutants and a number of clinical trials were launched using Onyx-015 for various cancer types including squamous-cell carcinoma of head and neck, and colorectal and ovarian cancers [Hamid, *et al.*, 2003; Hecht, *et al.*, 2003; Lamont, *et al.*, 2000; Makower, *et al.*, 2003; Nemunaitis, *et al.*, 2001; Nemunaitis, *et al.*, 2003; Post, 2002; Reid, *et al.*, 2002; Vasey, *et al.*, 2002]. However, the theory of P53 dependency for oncolytic replication of Onyx-015 has been challenged in the past few years [Dix, *et al.*, 2001] and it appears that the oncolytic properties of E1B deletion mutants may depend on many factors including other proteins associated with the P53 pathway, viral mRNA transport, higher CAR expression levels on tumor cells and variation in viral infectivity [Goodrum, *et al.*, 1998; Goodrum, *et al.*, 1999; Harada, *et al.*, 1999; Hutchin, *et al.*, 2000; Rothmann, *et al.*, 1998; Steegenga, *et al.*, 1999; Turnell, *et al.*, 1999]. Nevertheless, results of clinical trials on Onyx-015 have been encouraging. Patient treatments were all well tolerated in all trials including intravascular and intratumoral delivery [Reid, *et al.*, 2002] and there was some evidence of viral replication in the tumor cells with no virus detected in the surrounding tissue [Nemunaitis, *et al.*, 2001]. While virus treatment alone had only a mild efficacy, the combined use of cisplatin and

intratumoral ONYX-015 injection with cisplatin and 5-fluorouracil to treat patients with recurrent squamous cell cancer of the head and neck produced a good objective response [Khuri, *et al.*, 2000]. Furthermore, at 6 months, none of the responding tumors had progressed but all non-injected tumors treated with chemotherapy alone had. This trial has now advanced to phase III. E1A-CR2 deletion results in mutants such as dl922-947 that replicate at or above wild-type adenovirus levels in all tumor cells but several logs less efficiently in quiescent normal cells [Heise, *et al.*, 2000]. Replication of this type of mutants was significantly inhibited by Rb expression in Rb⁻ tumor cells [Fueyo, *et al.*, 2003; Suzuki, *et al.*, 2001]. It is interesting that E1A deletion mutants might be significantly more potent in oncolysis than E1B deletion mutants both *in vitro* and *in vivo* [Heise, *et al.*, 2000].

Selective viral replication can also be achieved by controlled viral gene expression at the transcriptional level. Regulatory regions of essential viral genes can be replaced with tumor cell-specific promoters to control gene expression solely in specific tumor cells. Some potential tumor-specific promoters have been listed in previous review articles [Galanis, *et al.*, 2001; McCormick, 2001]. A number of adenovirus vectors that utilise this strategy are currently in clinical trials. Adenoviruses CN706 [Rodriguez, *et al.*, 1997] and CV787 [Yu, *et al.*, 1999; Yu, *et al.*, 2001] have their E1A region controlled by the prostate-specific antigen (PSA) promoter and rat probasin promoter, respectively. The latter is also a prostate-specific promoter. It is worth mentioning that the promoters for PSA and probasin are active in normal prostate tissue. Therefore, the target vectors are tissue specific rather than tumor specific. More recently, the promoter of the human IAI.3B gene was isolated from ovarian cancer cells and a replication-selective adenovirus, AdE3-IAI.3B, driven by the promoter was created. The promoter activity of IAI.3B in ovarian cancer cells was almost the same as that of cytomegalovirus and an order of magnitude higher than those of midkine and cyclooxygenase-2. AdE3-IAI.3B replicated as efficiently as the wild-type adenovirus and caused extensive cell killing in an *in vitro* assay using a panel of ovarian cancer cells. In contrast, squamous cell carcinoma and normal cells were not able to support AdE3-IAI.3B replication. A strong therapeutic effect with this virus has been demonstrated in animal ovarian cancer models [Hamada, *et al.*, 2003].

HSV vectors are one of the earliest oncolytic viruses to be employed in clinical trials. However, due to the functional complexity of glycoproteins on the viral envelope, successful alteration of viral tropism remains a challenging task. To improve tumor specificity, control of viral replication at the transcriptional level is crucial. Miyatake *et al.* constructed a hepatoma-specific HSV vector (G92A) by inserting an albumin enhancer/promoter--ICP4 transgene into the thymidine kinase gene of a mutant HSV, in which both copies of native ICP4 genes were deleted. In human adults, albumin is expressed uniquely in the liver and in hepatocellular carcinoma, and is transcriptionally regulated. G92A was found to efficiently replicate *in vitro* in two human hepatoma cell lines expressing albumin, but not in four human non-hepatoma, albumin-non-expressing tumor cell lines, although all cell lines were equally susceptible to a

tissue nonspecific HSV recombinant, hrR3. In *in vivo* experiments, G92A replicated well in athymic mice transplanted with subcutaneous xenografts of human hepatoma cells (Hep3B), but not in those in which non-hepatoma subcutaneous tumors (PC3 and HeLa) were introduced, whereas hrR3 replicated well in both tumor types. Intratumoral inoculation of G92A inhibited the growth of established subcutaneous hepatoma tumors in nude mice, but not prostate tumors [Miyatake, *et al.*, 1999]. While this mutant has been used to demonstrate in principle that HSV virus can be regulated at a transcriptional level to enhance its tumor specificity, it is not suitable for clinical use. This is because viral replication of this mutant is controlled by a hepatoma promoter. Thus, normal liver cells will be not spared. Secondly, the transgene was inserted in the tk region, probably for the convenience of selection during construction of recombinant virus. Insertion in the tk region abolished the function of tk and rendered the mutant resistant to anti-herpetic drugs. Both shortcomings reduced the safety of this mutant for clinical application. A more recent recombinant, Myb34.5, has shown greater tumor specificity [Nakamura, *et al.*, 2002]. This mutant uses an ICP6 virus backbone but the endogenous promoter of the ICP34.5 gene has been replaced with the E2F-responsive cellular B-myc promoter [Chung, *et al.*, 1999]. ICP34.5 facilitates viral replication by promoting the dephosphorylation of eIF-2alpha. Thus, it blocks shut off of protein synthesis, which is an important host cell defence mechanism against the virus. Indeed, infection with Myb34.5 resulted in greater eIF-2alpha dephosphorylation and viral replication in colon carcinoma cells but not in normal hepatocytes. When administered intravascularly to mice with diffused liver metastases, Myb34.5 had greater antineoplastic activity than HSV-1 mutants with completely defective ICP34.5 expression and increased restriction of bio-distribution compared with HSV-1 mutants with wild-type ICP34.5 expression. Portal venous administration of Myb34.5 significantly reduced the tumor burden in the liver and prolonged the life of mice with diffused liver metastases.

Tumor-specific targeting by substituting a viral promoter with a tumor-specific cellular promoter may be influenced by the gene products produced by the rest of the viral genome. Since the genes of many viruses include standard polymerase II transcription units, exogenous polymerase II promoters used to express transgenes may be affected by viral gene products in the same way as are viral promoters. If the expression of a transgene could be augmented by viral transcriptional proteins, the specificity of tissue- or tumor-targeted therapy might thus be jeopardized. For instance, an HSV immediate early gene ICP0 has been shown to have a significant influence on transcriptional activities of both viral and cellular genes [Cheung, *et al.*, 1997; Jang, *et al.*, 1991; Kanangat, *et al.*, 1996; Margolis, *et al.*, 1992; Boutell, *et al.*, 2003; Sandri-Goldin, 2003]. Recently, we demonstrated that HSV ICP0 might override tumor/cell-specific promoters such as hTERT and tyrosinase promoter resulting in loss of promoter specificity [Yang, *et al.*, 2003]. In that study, we examined the activities of cellular promoters, including those for genes of human telomerase reverse transcriptase (hTERT), tyrosinase and probasin, in both tumor and normal cells after infection with HSV-1 vectors. Our results showed

that infection with replication-defective HSV-1 vectors significantly upregulated the activity of all three cellular promoters in a non-sequence-specific fashion in all cell types tested. Furthermore, viral infection upregulated activities of the hTERT promoter and endogenous telomerase in non-tumoral cells. Additional experiments suggested that viral ICPO may be responsible for the deregulation of cellular promoter activity and activation of telomerase since infection with ICPO⁻ mutants did not upregulate the activity of cellular promoters and since directly transfecting ICPO cDNA alone was sufficient to deregulate the specificity of these cellular promoters.

Tumor-specific viral replication can also be targeted at the translational level. One example is an intergeneric poliovirus [Gromeier, *et al.*, 2000; Gromeier, *et al.*, 2000]. Replication of poliovirus requires its internal ribosomal entry site (IRES) element for initiation of translation. The function of IRES in poliovirus is neuronal cell-type specific, which renders the virus neurovirulence. Replacing the poliovirus IRES with human rhinovirus type 2 resulted in a significantly attenuated neurovirulence. Interestingly, the recombinant poliovirus remained lytic and replicable in glioma cells [Gromeier, *et al.*, 2000]. This intergeneric recombinant poliovirus has shown non-pathogenic in primates and now in a clinical trial for treatment of gliomas.

WHERE IS THE FIELD GOING?

Gene therapy has been widely accepted as a valuable therapeutic concept. However, there is still a long way to go before we can apply knowledge acquired in the laboratory to the clinical treatment of patients. Incidences of adenovirus-caused patient death in 1999 and a recent retrovirus-caused leukemia-like disorder in gene therapy-treated SCID-XI children (for review, see) [Thomas, *et al.*, 2003] have added further turbulence to the ongoing development of this field. Viral-mediated gene therapy for treating cancer may pose less of a safety issue, as the duration of viral gene expression is relatively short. Moreover, the viral vectors currently used in clinical trials are probably so attenuated that efficient viral spreading among tumor cells is impeded, and this has been recognized as being one of the major obstacles to achieving good clinical benefits with this form of cancer treatment. Thus, in contrast to the field of gene therapy for non-tumor diseases, where safety has been a major concern, for virus mediated tumor treatment, clinical efficacy is the most upfront obstacle at the present time.

To enhance the efficacy, more virulent oncolytic viruses are required. Since levels of virulence of wild type viruses vary significantly, some strains are less infectious or replicative than others. Unfortunately, most of the current viral vectors were based on a few wild-type strains that were only mildly virulent, either due to many generations of passage in a laboratory environment or because they were simply mild at the outset. It may be important to compare wild-type viral backbones for their lytic efficiency in animal tumor models prior to developing vectors for gene therapy.

To improve construction of oncolytic vectors, an increased understanding of the basic molecular biology of the viral lytic cycle machinery is required. The expression of viral genes occurs as a well-orchestrated series of events

with a strict temporal and quantitative pattern. In addition, most viral genes/proteins are multifunctional. Thus, deletion or modification of any one of the viral genes may result in unwanted changes in other functions that could affect the efficiency of viral replication. Therefore, more sophisticated forms of genetic modification of the viral genome are needed that are based on a more in depth and broader understanding of molecular virology.

The safety of the more virulent viral vectors must be ensured by enhanced tumor-specificity. Altering the viral tropism through modification of viral surface molecules would be the first choice since increasing affinity of virions to tumor cells vs. normal cells would provide the agent with an optimal bio-distribution and pharmacokinetics profile and a lower dose of virus particles would need to be administered. Furthermore, specific targeting of tumor cells through recognition of surface molecules makes intravenous injection a safe and efficient means of delivering the vectors to metastatic tumors. The second choice for tumor targeting will be to alter the efficiency of viral replication in such a way that virus growth is favored in tumor cells, but not in normal cells. This can be achieved by regulating essential viral gene expression at both transcriptional and translation levels. This approach would only be a second choice as a tumor-specific targeting strategy as it does not change the initial bio-distribution of the viral vectors in the body, which will mean that much healthy tissue can be infected and bear the risk of virus-induced toxicity. Eventually, however, the largest portion of the viral vectors should be concentrated in the tumor due to altered efficiency in viral replication among the various tissues. Of course, strategies based on re-directed viral tropism and selective replication can be combined as they have been with several recombinant adenovirus vectors [Nagi, *et al.*, 2003].

Finally, oncolytic viral vectors may carry therapeutic genes to enhance their efficacy. Diffusible gene products can especially complement an incomplete viral coverage in a tumor mass. On the other hand, enhanced viral dissemination in tumor mass by oncolytic viral vectors will facilitate the distribution of the gene product in the tumor mass. However, one must be careful to select the optimal type of transgene and the most appropriate time for its expression in order that the propagation of virus in the tumor is not jeopardized. For instance, cytotoxic genes with diffusible products may hinder spread of virus in the tumor mass. The concentration gradient of toxic molecules released from the infected cells will kill the surrounding cells first, which will block virus propagation. To avoid a potential conflict in tumor between cytotoxicity and viral spread, prodrug genes such as the hsv-tk gene, the cytosine deaminase and the nitroreductase gene etc. [Chen, *et al.*, 2002; Kim, *et al.*, 2002; Yazawa, *et al.*, 2002] can be used. Alternatively, cytotoxic genes may be controlled by an inducible promoter or a late virus promoter [Sauthoff, *et al.*, 2002]. Both strategies can hold on the cytotoxic effect of inserted gene until the virus has fully disseminated in the tumor mass.

Oncolytic viruses may represent one of the most promising treatments for malignant tumors. Viral lytic replication is highly efficient by playing two roles simultaneously; destruction of tumor cells and dissemination

of virus itself inside of tumor mass. Furthermore, viruses lyse tumor cells through multiple mechanisms, without depending on a specific pathway. This strategy has its unique advantage given the heterogenous nature of cancer cells in a tumor mass. Biological complicity of viruses provides many opportunities for manipulation to create new recombinants with high efficiency and specificity for tumor destruction.

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REFERENCES

- Aghi, M., Hochberg, F., Breakefield, X.O. (2000) Prodrug activation enzymes in cancer gene therapy. *J Gene Med*, **2**:148-64.
- Akiyama, Y., Maruyama, K., Watanabe, M., Yamaguchi, K. (2002) Retroviral-mediated IL-12 gene transduction into human CD34+ cell-derived dendritic cells. *Int J Oncol*, **21**:509-14.
- Arafat, W.O., Casado, E., Wang, M., Alvarez, R.D., Siegal, G.P., Glorioso, J.C., Curiel, D.T., Gomez-Navarro, J. (2000) Genetically modified CD34+ cells exert a cytotoxic bystander effect on human endothelial and cancer cells. *Clin Cancer Res*, **6**:4442-8.
- Bell, J.C., Lichty, B., Stojdl, D. (2003) Getting oncolytic virus therapies off the ground. *Cancer Cell*, **4**:7-11.
- Bender, F.C., Whitbeck, J.C., Ponce de Leon, M., Lou, H., Eisenberg, R.J., Cohen, G.H. (2003) Specific association of glycoprotein B with lipid rafts during herpes simplex virus entry. *J Virol*, **77**:9542-52.
- Bennett, J. (2003) Immune response following intraocular delivery of recombinant viral vectors. *Gene Ther*, **10**:977-82.
- Bischoff, J.R., Kim, D.H., Williams, A., Heise, C., Horn, S., Muna, M., Ng, L., Nye, J.A., Sampson-Johannes, A., Fattaey, A., McCormick, F. (1996) An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science*, **274**:373-6.
- Boutell, C., Everett, R.D. (2003) The herpes simplex virus type 1 (HSV-1) regulatory protein ICP0 interacts with and Ubiquitinates p53. *J Biol Chem*, **278**:36596-602.
- Boviatsis, E.J., Chase, M., Wei, M.X., Tamiya, T., Hurford, R.K. Jr., Kowall, N.W., Tepper, R.I., Breakefield, X.O., Chioccia, E.A. (1994) Gene transfer into experimental brain tumors mediated by adenovirus, herpes simplex virus, and retrovirus vectors. *Hum Gene Ther*, **5**:183-91.
- Burton, E.A., Fink, D.J., Glorioso, J.C. (2002) Gene delivery using herpes simplex virus vectors. *DNA Cell Biol*, **21**:915-36.
- Chen, D., Murphy, B., Sung, R., Bromberg, J.S. (2003) Adaptive and innate immune responses to gene transfer vectors: role of cytokines and chemokines in vector function. *Gene Ther*, **10**:991-8.
- Chen, L., Waxman, D.J. (2002) Cytochrome P450 gene-directed enzyme prodrug therapy (GDEPT) for cancer. *Curr Pharm Des*, **8**:1405-16.
- Cheney, I.W., Johnson, D.E., Vaillancourt, M.T., Avanzini, J., Morimoto, A., Demers, G.W., Wills, K.N., Shabram, P.W., Bolen, J.B., Tavtigian, S.V., Bookstein, R. (1998) Suppression of tumorigenicity of glioblastoma cells by adenovirus-mediated MMAC1/PTEN gene transfer. *Cancer Res*, **58**:2331-4.
- Cheshenko, N., Herold, B.C. (2002) Glycoprotein B plays a predominant role in mediating herpes simplex virus type 2 attachment and is required for entry and cell-to-cell spread. *J Gen Virol*, **83**:2247-55.
- Cheung, P., Panning, B., Smiley, J.R. (1997) Herpes simplex virus immediate-early proteins ICP0 and ICP4 activate the endogenous human alpha-globin gene in nonerythroid cells. *J Virol*, **71**:1784-93.
- Chung, R.Y., Saeki, Y., Chioccia, E.A. (1999) B-myb promoter retargeting of herpes simplex virus gamma34.5 gene-mediated virulence toward tumor and cycling cells. *J Virol*, **73**:7556-64.
- Cohen, E.E., Rudin, C.M. (2001) ONYX-015. Onyx Pharmaceuticals. *Curr Opin Investig Drugs*, **2**:1770-5.
- Cornelis, J.J., Chen, Y.Q., Spruyt, N., Duponchel, N., Cotmore, S.F., Tattersall, P., Rommelaere, J. (1990) Susceptibility of human cells to killing by the parvoviruses H-1 and minute virus of mice correlates with viral transcription. *J Virol*, **64**:2537-44.
- Culver, K.W., Ram, Z., Wallbridge, S., Ishii, H., Oldfield, E.H., Blaese, R.M. (1992) *In vivo* gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. *Science*, **256**:1550-2.
- Curiel, D.T. (1999) Strategies to adapt adenoviral vectors for targeted delivery. *Ann N Y Acad Sci*, **886**:158-71.
- Dix, B.R., Edwards, S.J., Braithwaite, A.W. (2001) Does the antitumor adenovirus ONYX-015/d11520 selectively target cells defective in the p53 pathway? *J Virol*, **75**:5443-7.
- Dobbelstein, M. (2003) Viruses in therapy--royal road or dead end? *Virus Res*, **92**:219-21.
- Douglas, J.T., Miller, C.R., Kim, M., Dmitriev, I., Mikheeva, G., Krasnykh, V., Curiel, D.T. (1999) A system for the propagation of adenoviral vectors with genetically modified receptor specificities. *Nat Biotechnol*, **17**:470-5.
- Ebara, S., Shimura, S., Nasu, Y., Kaku, H., Kumon, H., Yang, G., Wang, J., Timme, T.L., Aguilar-Cordova, E., Thompson, T.C. (2002) Gene therapy for prostate cancer: toxicological profile of four HSV-tk transducing adenoviral vectors regulated by different promoters. *Prostate Cancer Prostatic Dis*, **5**:316-25.
- Ferrari, S., Griesenbach, U., Geddes, D.M., Alton, E. (2003) Immunological hurdles to lung gene therapy. *Clin Exp Immunol*, **132**:1-8.
- Fillat, C., Carrio, M., Cascante, A., Sangro, B. (2003) Suicide gene therapy mediated by the Herpes Simplex virus thymidine kinase gene/Ganciclovir system: fifteen years of application. *Curr Gene Ther*, **3**:13-26.
- Fontana, L., Nuzzo, M., Urbanelli, L., Monaci, P. (2003) General strategy for broadening adenovirus tropism. *J Virol*, **77**:11094-104.
- Fueyo, J., Alemany, R., Gomez-Manzano, C., Fuller, G.N., Khan, A., Conrad, C.A., Liu, T.J., Jiang, H., Lemoine, M.G., Suzuki, K., Sawaya, R., Curiel, D.T., et al. (2003) Preclinical characterization of the antiangioma activity of a tropism-enhanced adenovirus targeted to the retinoblastoma pathway. *J Natl Cancer Inst*, **95**:652-60.
- Galanis, E., Vile, R., Russell, S.J. (2001) Delivery systems intended for *in vivo* gene therapy of cancer: targeting and replication competent viral vectors. *Crit Rev Oncol Hematol*, **38**:177-92.
- Goldstein, D.J., Weller, S.K. (1988) Herpes simplex virus type 1-induced ribonucleotide reductase activity is dispensable for virus growth and DNA synthesis: isolation and characterization of an ICP6 lacZ insertion mutant. *J Virol*, **62**:196-205.
- Gomella, L.G., Mastrangelo, M.J., McCue, P.A., Maguire, H.J., Mulholland, S.G., Lattime, E.C. (2001) Phase I study of intravesical vaccinia virus as a vector for gene therapy of bladder cancer. *J Urol*, **166**:1291-5.
- Goodrum, F.D., Ornelles, D.A. (1998) p53 status does not determine outcome of E1B 55-kilodalton mutant adenovirus lytic infection. *J Virol*, **72**:9479-90.
- Goodrum, F.D., Ornelles, D.A. (1999) Roles for the E4 orf6, orf3, and E1B 55-kilodalton proteins in cell cycle-independent adenovirus replication. *J Virol*, **73**:7474-88.
- Gromeier, M., Lachmann, S., Rosenfeld, M.R., Gutin, P.H., Wimmer, E. (2000) Intergenic poliovirus recombinants for the treatment of malignant glioma. *Proc Natl Acad Sci U S A*, **97**:6803-8.
- Gromeier, M., Solecki, D., Patel, D.D., Wimmer, E. (2000) Expression of the human poliovirus receptor/CD155 gene during development of the central nervous system: implications for the pathogenesis of poliomyelitis. *Virology*, **273**:248-57.
- Guan, J., Ma, L., Wei, L. (2001) Characteristics of ovarian cancer cells transduced by the bicistronic retroviral vector containing GM-CSF and HSV-TK genes. *Chin Med J (Engl)*, **114**:147-51.
- Hall, M.C., Li, Y., Pong, R.C., Ely, B., Sagalowsky, A.I., Hsieh, J.T. (2000) The growth inhibitory effect of p21 adenovirus on human bladder cancer cells. *J Urol*, **163**:1033-8.
- Hamada, K., Kohno, S., Iwamoto, M., Yokota, H., Okada, M., Tagawa, M., Hirose, S., Yamasaki, K., Shirakata, Y., Hashimoto, K., Ito, M. (2003) Identification of the human IAI.3B promoter element and its use in the construction of a replication-selective adenovirus for ovarian cancer therapy. *Cancer Res*, **63**:2506-12.
- Hamid, O., Varterasian, M.L., Wadler, S., Hecht, J.R., Benson, A., 3rd, Galanis, E., Uprichard, M., Omer, C., Bycott, P., Hackman, R.C., Shields, A.F. (2003) Phase II trial of intravenous CI-1042 in patients with metastatic colorectal cancer. *J Clin Oncol*, **21**:1498-504.
- Harada, J.N., Berk, A.J. (1999) p53-Independent and -dependent requirements for E1B-55K in adenovirus type 5 replication. *J Virol*, **73**:5333-44.
- Hashiro, G., Loh, P.C., Yau, J.T. (1977) The preferential cytotoxicity of reovirus for certain transformed cell lines. *Arch Virol*, **54**:307-15.
- Hecht, J.R., Bedford, R., Abbruzzese, J.L., Lahoti, S., Reid, T.R., Soetikno, R.M., Kim, D.H., Freeman, S.M. (2003) A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with

- intravenous gemcitabine in unresectable pancreatic carcinoma. *Clin Cancer Res*, **9**:555-61.
- Heise, C., Hermiston, T., Johnson, L., Brooks, G., Sampson-Johannes, A., Williams, A., Hawkins, L., Kirn, D. (2000) An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. *Nat Med*, **6**:1134-9.
- Hemmati, P.G., Gillissen, B., von Haefen, C., Wendt, J., Starck, L., Guner, D., Dorken, B., Daniel, P.T. (2002) Adenovirus-mediated overexpression of p14(ARF) induces p53 and Bax-independent apoptosis. *Oncogene*, **21**:3149-61.
- Hirschowitz, E., Hidalgo, G., Doherty, D. (2002) Induction of cyclooxygenase-2 in non-small cell lung cancer cells by infection with DeltaE1, DeltaE3 recombinant adenovirus vectors. *Gene Ther*, **9**:81-4.
- Hlavaty, J., Tyukosova, S., Bies, J., Hlubinova, K., Altaner, C. (2000) Retrovirus vector containing wild type p53 gene and its effect on human glioma cells. *Neoplasma*, **47**:204-11.
- Huh, W.K., Gomez-Navarro, J., Arafat, W.O., Xiang, J., Mahasreshthi, P.J., Alvarez, R.D., Barnes, M.N., Curiel, D.T. (2001) Bax-induced apoptosis as a novel gene therapy approach for carcinoma of the cervix. *Gynecol Oncol*, **83**:370-7.
- Hutchin, M.E., Pickles, R.J., Yarbrough, W.G. (2000) Efficiency of adenovirus-mediated gene transfer to oropharyngeal epithelial cells correlates with cellular differentiation and human coxsackie and adenovirus receptor expression. *Hum Gene Ther*, **11**:2365-75.
- Jang, K.L., Pulverer, B., Woodgett, J.R., Latchman, D.S. (1991) Activation of the cellular transcription factor AP-1 in herpes simplex virus infected cells is dependent on the viral immediate-early protein ICPO. *Nucleic Acids Res*, **19**:4879-83.
- Jia, W.W., McDermott, M., Goldie, J., Cynader, M., Tan, J., Tufaro, F. (1994) Selective destruction of gliomas in immunocompetent rats by thymidine kinase-defective herpes simplex virus type 1. *J Natl Cancer Inst*, **86**:1209-15.
- Jooss, K., Chirmule, N. (2003) Immunity to adenovirus and adeno-associated viral vectors: implications for gene therapy. *Gene Ther*, **10**:955-63.
- Kanangat, S., Babu, J.S., Knipe, D.M., Rouse, B.T. (1996) HSV-1-mediated modulation of cytokine gene expression in a permissive cell line: selective upregulation of IL-6 gene expression. *Virology*, **219**:295-300.
- Katner, A.L., Gootam, P., Hoang, Q.B., Gnarra, J.R., Rayford, W. (2002) A recombinant adenovirus expressing p7(Kip1) induces cell cycle arrest and apoptosis in human 786-0 renal carcinoma cells. *J Urol*, **168**:766-73.
- Khuri, F.R., Nemunaitis, J., Ganly, I., Arseneau, J., Tannock, I.F., Romel, L., Gore, M., Ironside, J., MacDougall, R.H., Heise, C., Randlev, B., Gillenwater, A.M., et al. (2000) A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med*, **6**:879-85.
- Kirn, D., Niculescu-Duvaz, I., Hallden, G., Springer, C.J. (2002) The emerging fields of suicide gene therapy and virotherapy. *Trends Mol Med*, **8**:S68-73.
- Kleeff, J., Fukahi, K., Lopez, M.E., Friess, H., Buchler, M.W., Sosnowski, B.A., Korc, M. (2002) Targeting of suicide gene delivery in pancreatic cancer cells via FGF receptors. *Cancer Gene Ther*, **9**:522-32.
- Kubo, H., Gardner, T.A., Wada, Y., Koeneman, K.S., Gotoh, A., Yang, L., Kao, C., Lim, S.D., Amin, M.B., Yang, H., Black, M.E., Matsubara, S., et al. (2003) Phase I dose escalation clinical trial of adenovirus vector carrying osteocalcin promoter-driven herpes simplex virus thymidine kinase in localized and metastatic hormone-refractory prostate cancer. *Hum Gene Ther*, **14**:227-41.
- Lamont, J.P., Nemunaitis, J., Kuhn, J.A., Landers, S.A., McCarty, T.M. (2000) A prospective phase II trial of ONYX-015 adenovirus and chemotherapy in recurrent squamous cell carcinoma of the head and neck (the Baylor experience). *Ann Surg Oncol*, **7**:588-92.
- Liu, Q., Muruve, D.A. (2003) Molecular basis of the inflammatory response to adenovirus vectors. *Gene Ther*, **10**:935-40.
- Lorence, R.M., Rood, P.A., Kelley, K.W. (1988) Newcastle disease virus as an antineoplastic agent: induction of tumor necrosis factor-alpha and augmentation of its cytotoxicity. *J Natl Cancer Inst*, **80**:1305-12.
- Lowenstein, P.R., Castro, M.G. (2003) Inflammation and adaptive immune responses to adenoviral vectors injected into the brain: peculiarities, mechanisms, and consequences. *Gene Ther*, **10**:946-54.
- Lundstrom, K. (2002) Alphavirus vectors as tools in cancer gene therapy. *Technol Cancer Res Treat*, **1**:83-8.
- Lundstrom, K. (2003) Latest development in viral vectors for gene therapy. *Trends Biotechnol*, **21**:117-22.
- Ma, H.I., Lin, S.Z., Chiang, Y.H., Li, J., Chen, S.L., Tsao, Y.P., Xiao, X. (2002) Intratumoral gene therapy of malignant brain tumor in a rat model with angiostatin delivered by adeno-associated viral (AAV) vector. *Gene Ther*, **9**:2-11.
- Makower, D., Rozenblit, A., Kaufman, H., Edelman, M., Lane, M.E., Zwiebel, J., Haynes, H., Wadler, S. (2003) Phase II clinical trial of intralesional administration of the oncolytic adenovirus ONYX-015 in patients with hepatobiliary tumors with correlative p53 studies. *Clin Cancer Res*, **9**:693-702.
- Mardberg, K., Trybala, E., Tufaro, F., Bergstrom, T. (2002) Herpes simplex virus type 1 glycoprotein C is necessary for efficient infection of chondroitin sulfate-expressing gro2C cells. *J Gen Virol*, **83**:291-300.
- Margolis, D.M., Rabson, A.B., Straus, S.E., Ostrove, J.M. (1992) Transactivation of the HIV-1 LTR by HSV-1 immediate-early genes. *Virology*, **186**:788-91.
- Markert, J.M., Medlock, M.D., Rabkin, S.D., Gillespie, G.Y., Todo, T., Hunter, W.D., Palmer, C.A., Feigenbaum, F., Tornatore, C., Tufaro, F., Martuza, R.L. (2000) Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther*, **7**:867-74.
- Martuza, R.L., Mallick, A., Markert, J.M., Ruffner, K.L., Coen, D.M. (1991) Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science*, **252**:854-6.
- Matsumoto, G., Shindo, J. (2001) Cancer therapy by gene therapy with angiostatin. *Drugs Today (Barc)*, **37**:815-21.
- Maxwell, I.H., Terrell, K.L., Maxwell, F. (2002) Autonomous parvovirus vectors. *Methods*, **28**:168-81.
- Mazzolini, G., Prieto, J., Melero, I. (2003) Gene therapy of cancer with interleukin-12. *Curr Pharm Des*, **9**:1981-91.
- McCormick, F. (2001) Cancer gene therapy: fringe or cutting edge? *Nat Rev Cancer*, **1**:130-41.
- Merritt, J.A., Roth, J.A., Logothetis, C.J. (2001) Clinical evaluation of adenoviral-mediated p53 gene transfer: review of INGN 201 studies. *Semin Oncol*, **28**:105-14.
- Michael, S.I., Hong, J.S., Curiel, D.T., Engler, J.A. (1995) Addition of a short peptide ligand to the adenovirus fiber protein. *Gene Ther*, **2**:660-8.
- Miyatake, S.I., Tani, S., Feigenbaum, F., Sundaresan, P., Toda, H., Narumi, O., Kikuchi, H., Hashimoto, N., Hangai, M., Martuza, R.L., Rabkin, S.D. (1999) Hepatoma-specific antitumor activity of an albumin enhancer/promoter regulated herpes simplex virus *in vivo*. *Gene Ther*, **6**:564-72.
- Nagi, P., Vickers, S.M., Davydova, J., Adachi, Y., Takayama, K., Barker, S., Krasnykh, V., Curiel, D.T., Yamamoto, M. (2003) Development of a therapeutic adenoviral vector for cholangiocarcinoma combining tumor-restricted gene expression and infectivity enhancement. *J Gastrointest Surg*, **7**:364-71.
- Nakamura, H., Kasuya, H., Mullen, J.T., Yoon, S.S., Pawlik, T.M., Chandrasekhar, S., Donahue, J.M., Chiocca, E.A., Chung, R.Y., Tanabe, K.K. (2002) Regulation of herpes simplex virus gamma(1)34.5 expression and oncolysis of diffuse liver metastases by Myb34.5. *J Clin Invest*, **109**:871-82.
- Nemunaitis, J., Khuri, F., Ganly, I., Arseneau, J., Posner, M., Vokes, E., Kuhn, J., McCarty, T., Landers, S., Blackburn, A., Romel, L., Randlev, B., et al. (2001) Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J Clin Oncol*, **19**:289-98.
- Nemunaitis, J., Cunningham, C., Tong, A.W., Post, L., Netto, G., Paulson, A.S., Rich, D., Blackburn, A., Sands, B., Gibson, B., Randlev, B., Freeman, S. (2003) Pilot trial of intravenous infusion of a replication-selective adenovirus (ONYX-015) in combination with chemotherapy or IL-2 treatment in refractory cancer patients. *Cancer Gene Ther*, **10**:341-52.
- Okabe, S., Arai, T., Yamashita, H., Sugihara, K. (2003) Adenovirus-mediated prodrug-enzyme therapy for CEA-producing colorectal cancer cells. *J Cancer Res Clin Oncol*, **129**:367-73.
- Park, K.H., Kim, G., Jang, S.H., Kim, C.H., Kwon, S.Y., Yoo, C.G., Kim, Y.W., Kwon, H.C., Kim, C.M., Han, S.K., Shim, Y.S., Lee, C.T. (2003) Gene therapy with GM-CSF, interleukin-4 and herpes simplex virus thymidine kinase shows strong antitumor effect on lung cancer. *Anticancer Res*, **23**:1559-64.
- Pecora, A.L., Rizvi, N., Cohen, G.I., Meropol, N.J., Serman, D., Marshall, J.L., Goldberg, S., Gross, P., O'Neil, J.D., Groene, W.S., Roberts, M.S., Rabin, H. (2002) Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. *J Clin Oncol*, **20**:2251-66.

- Post, L.E. (2002) Selectively replicating adenoviruses for cancer therapy: an update on clinical development. *Curr Opin Investig Drugs*, **3**:1768-72.
- Rainov, N.G. (2000) A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum Gene Ther*, **11**:2389-401.
- Rajcani, J., Vojvodova, A. (1998) The role of herpes simplex virus glycoproteins in the virus replication cycle. *Acta Virol*, **42**:103-18.
- Reid, T., Warren, R., Kirn, D. (2002) Intravascular adenoviral agents in cancer patients: lessons from clinical trials. *Cancer Gene Ther*, **9**:979-86.
- Ring, C.J. (2002) Cytolytic viruses as potential anti-cancer agents. *J Gen Virol*, **83**:491-502.
- Rochlitz, C., Figlin, R., Squiban, P., Salzberg, M., Pless, M., Herrmann, R., Tartour, E., Zhao, Y., Bizouarne, N., Baudin, M., Acres, B. (2003) Phase I immunotherapy with a modified vaccinia virus (MVA) expressing human MUC1 as antigen-specific immunotherapy in patients with MUC1-positive advanced cancer. *J Gene Med*, **5**:690-9.
- Rodriguez, R., Schuur, E.R., Lim, H.Y., Henderson, G.A., Simons, J.W., Henderson, D.R. (1997) Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Res*, **57**:2559-63.
- Rogers, B.E., Douglas, J.T., Ahlem, C., Buchsbaum, D.J., Frincke, J., Curiel, D.T. (1997) Use of a novel cross-linking method to modify adenovirus tropism. *Gene Ther*, **4**:1387-92.
- Roizman, B. (1996) The function of herpes simplex virus genes: a primer for genetic engineering of novel vectors. *Proc Natl Acad Sci USA*, **93**:11307-12.
- Rommelaere, J., Cornelis, J.J. (1991) Antineoplastic activity of parvoviruses. *J Virol Methods*, **33**:233-51.
- Roth, J.A., Swisher, S.G., Merritt, J.A., Lawrence, D.D., Kemp, B.L., Carrasco, C.H., El-Naggar, A.K., Fossella, F.V., Glisson, B.S., Hong, W.K., Khurl, F.R., Kurie, J.M. (1998) Gene therapy for non-small cell lung cancer: a preliminary report of a phase I trial of adenoviral p53 gene replacement. *Semin Oncol*, **25**:33-7.
- Rothmann, T., Hengstermann, A., Whitaker, N.J., Scheffner, M., zur Hausen, H. (1998) Replication of ONYX-015, a potential anticancer adenovirus, is independent of p53 status in tumor cells. *J Virol*, **72**:9470-8.
- Rots, M.G., Curiel, D.T., Gerritsen, W.R., Haisma, H.J. (2003) Targeted cancer gene therapy: the flexibility of adenoviral gene therapy vectors. *J Control Release*, **87**:159-65.
- Rux, A.H., Lou, H., Lambris, J.D., Friedman, H.M., Eisenberg, R.J., Cohen, G.H. (2002) Kinetic analysis of glycoprotein C of herpes simplex virus types 1 and 2 binding to heparin, heparan sulfate, and complement component C3b. *Virology*, **294**:324-32.
- Ruzek, M.C., Kavanagh, B.F., Scaria, A., Richards, S.M., Garman, R.D. (2002) Adenoviral vectors stimulate murine natural killer cell responses and demonstrate antitumor activities in the absence of transgene expression. *Mol Ther*, **5**:115-24.
- Sandri-Goldin, R.M. (2003) Replication of the herpes simplex virus genome: does it really go around in circles? *Proc Natl Acad Sci U S A*, **100**:7428-9.
- Sauthoff, H., Pipiya, T., Heitner, S., Chen, S., Norman, R.G., Rom, W.N., Hay, J.G. (2002) Late expression of p53 from a replicating adenovirus improves tumor cell killing and is more tumor cell specific than expression of the adenoviral death protein. *Hum Gene Ther*, **13**:1859-71.
- Sauthoff, H., Hu, J., Maca, C., Goldman, M., Heitner, S., Yee, H., Pipiya, T., Rom, W.N., Hay, J.G. (2003) Intratumoral spread of wild-type adenovirus is limited after local injection of human xenograft tumors: virus persists and spreads systemically at late time points. *Hum Gene Ther*, **14**:425-33.
- Schneider, U., Bullough, F., Vongpunawad, S., Russell, S.J., Cattaneo, R. (2000) Recombinant measles viruses efficiently entering cells through targeted receptors. *J Virol*, **74**:9928-36.
- Schnell, M.J., Buonocore, L., Kretzschmar, E., Johnson, E., Rose, J.K. (1996) Foreign glycoproteins expressed from recombinant vesicular stomatitis viruses are incorporated efficiently into virus particles. *Proc Natl Acad Sci U S A*, **93**:11359-65.
- Shi, W., Teschendorf, C., Muzyczka, N., Siemann, D.W. (2002) Adeno-associated virus-mediated gene transfer of endostatin inhibits angiogenesis and tumor growth *in vivo*. *Cancer Gene Ther*, **9**:513-21.
- Shimada, H., Matsubara, H., Ochiai, T. (2002) p53 gene therapy for esophageal cancer. *J Gastroenterol*, **37 Suppl 14**:87-91.
- St George, J.A. (2003) Gene therapy progress and prospects: adenoviral vectors. *Gene Ther*, **10**:1135-41.
- Steegenga, W.T., Riteco, N., Bos, J.L. (1999) Infectivity and expression of the early adenovirus proteins are important regulators of wild-type and DeltaE1B adenovirus replication in human cells. *Oncogene*, **18**:5032-43.
- Steiner, M.S., Zhang, Y., Farooq, F., Lerner, J., Wang, Y., Lu, Y. (2000) Adenoviral vector containing wild-type p16 suppresses prostate cancer growth and prolongs survival by inducing cell senescence. *Cancer Gene Ther*, **7**:360-72.
- Stojdl, D.F., Abraham, N., Knowles, S., Marius, R., Brasey, A., Lichty, B.D., Brown, E.G., Sonenberg, N., Bell, J.C. (2000) The murine double-stranded RNA-dependent protein kinase PKR is required for resistance to vesicular stomatitis virus. *J Virol*, **74**:9580-5.
- Stojdl, D.F., Lichty, B., Knowles, S., Marius, R., Atkins, H., Sonenberg, N., Bell, J.C. (2000) Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat Med*, **6**:821-5.
- Strong, J.E., Coffey, M.C., Tang, D., Sabinin, P., Lee, P.W. (1998) The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. *EMBO J*, **17**:3351-62.
- Sutton, M.A., Freund, C.T., Berkman, S.A., Dang, T.D., Kattan, M.W., Wheeler, T.M., Rowley, D.R., Lerner, S.P. (2000) *In vivo* adenovirus-mediated suicide gene therapy of orthotopic bladder cancer. *Mol Ther*, **2**:211-7.
- Suzuki, K., Fueyo, J., Krasnykh, V., Reynolds, P.N., Curiel, D.T., Alemany, R. (2001) A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. *Clin Cancer Res*, **7**:120-6.
- Tanaka, T., Manome, Y., Wen, P., Kufe, D.W., Fine, H.A. (1997) Viral vector-mediated transduction of a modified platelet factor 4 cDNA inhibits angiogenesis and tumor growth. *Nat Med*, **3**:437-42.
- Tanaka, T., Cao, Y., Folkman, J., Fine, H.A. (1998) Viral vector-targeted antiangiogenic gene therapy utilizing an angiostatin complementary DNA. *Cancer Res*, **58**:3362-9.
- Thomas, C.E., Ehrhardt, A., Kay, M.A. (2003) Progress and problems with the use of viral vectors for gene therapy. *Nat Rev Genet*, **4**:346-58.
- Timiryasova, T.M., Chen, B., Fodor, I. (2001) Replication-deficient vaccinia virus gene therapy vector: evaluation of exogenous gene expression mediated by PUV-inactivated virus in glioma cells. *J Gene Med*, **3**:468-77.
- Trudel, S., Trachtenberg, J., Toi, A., Sweet, J., Hua Li, Z., Jewett, M., Tshilias, J., Zhuang, L.H., Hitt, M., Wan, Y., Gauldie, J., Graham, F.L. (2003) A phase I trial of adenovector-mediated delivery of interleukin-2 (AdIL-2) in high-risk localized prostate cancer. *Cancer Gene Ther*, **10**:755-63.
- Turnell, A.S., Grand, R.J., Gallimore, P.H. (1999) The replicative capacities of large E1B-null group A and group C adenoviruses are independent of host cell p53 status. *J Virol*, **73**:2074-83.
- Turturro, F. (2003) Recombinant adenovirus-mediated cytotoxic gene therapy of lymphoproliferative disorders: is CAR important for the vector to ride? *Gene Ther*, **10**:100-4.
- Ueda, K., Iwahashi, M., Nakamori, M., Nakamura, M., Matsuura, I., Ojima, T., Yamaue, H. (2003) Improvement of carcinoembryonic antigen-specific prodrug gene therapy for experimental colon cancer. *Surgery*, **133**:309-17.
- Vasey, P.A., Shulman, L.N., Campos, S., Davis, J., Gore, M., Johnston, S., Kirn, D.H., O'Neill, V., Siddiqui, N., Seiden, M.V., Kaye, S.B. (2002) Phase I trial of intraperitoneal injection of the E1B-55-kd-gene-deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer. *J Clin Oncol*, **20**:1562-9.
- Wakimoto, H., Johnson, P.R., Knipe, D.M., Chiocca, E.A. (2003) Effects of innate immunity on herpes simplex virus and its ability to kill tumor cells. *Gene Ther*, **10**:983-90.
- Whitehouse, A. (2003) Herpesvirus saimiri: a potential gene delivery vector (review). *Int J Mol Med*, **11**:139-48.
- Wickham, T.J., Roelvink, P.W., Brough, D.E., Kovacs, I. (1996) Adenovirus targeted to heparan-containing receptors increases its gene delivery efficiency to multiple cell types. *Nat Biotechnol*, **14**:1570-3.
- Wierdl, M., Morton, C.L., Weeks, J.K., Danks, M.K., Harris, L.C., Potter, P.M. (2001) Sensitization of human tumor cells to CPT-11 via adenoviral-mediated delivery of a rabbit liver carboxylesterase. *Cancer Res*, **61**:5078-82.
- Wilson, D.R. (2002) Viral-mediated gene transfer for cancer treatment. *Curr Pharm Biotechnol*, **3**:151-64.

- Witte, O.N., Baltimore, D. (1977) Mechanism of formation of pseudotypes between vesicular stomatitis virus and murine leukemia virus. *Cell*, **11**:505-11.
- Wu, Q., Moyana, T., Xiang, J. (2001) Cancer gene therapy by adenovirus-mediated gene transfer. *Curr Gene Ther*, **1**:101-22.
- Yang, C.T., Song, J., Bu, X., Cong, Y.S., Bacchetti, S., Rennie, P., Jia, W.W. (2003) Herpes simplex virus type-1 infection upregulates cellular promoters and telomerase activity in both tumor and nontumor human cells. *Gene Ther*, **10**:1494-502.
- Yazawa, K., Fisher, W.E., Brunicardi, F.C. (2002) Current progress in suicide gene therapy for cancer. *World J Surg*, **26**:783-9.
- Yu, D.C., Chen, Y., Seng, M., Dilley, J., Henderson, D.R. (1999) The addition of adenovirus type 5 region E3 enables calydon virus 787 to eliminate distant prostate tumor xenografts. *Cancer Res*, **59**:4200-3.
- Yu, D.C., Chen, Y., Dilley, J., Li, Y., Embry, M., Zhang, H., Nguyen, N., Amin, P., Oh, J., Henderson, D.R. (2001) Antitumor synergy of CV787, a prostate cancer-specific adenovirus, and paclitaxel and docetaxel. *Cancer Res*, **61**:517-25.
- Zhang, M., Li, S., Nyati, M.K., DeRemer, S., Parsels, J., Rehemtulla, A., Ensminger, W.D., Lawrence, T.S. (2003) Regional delivery and selective expression of a high-activity yeast cytosine deaminase in an intrahepatic colon cancer model. *Cancer Res*, **63**:658-63.