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Ronald G. Crystal

In vivo and ex vivo gene therapy strategies to treat tumors using adenovirus gene transfer vectors

Abstract The adaptation of gene therapy strategies to treat tumors has broadened the potential armamentarium of anticancer strategies to include approaches for local control of tumor growth as well as to enhance systemic antitumor immunity to treat metastases. A major focus of the author and colleagues has been to use replication-deficient adenovirus vectors, both in vivo and ex vivo, to enhance local control of and systemic immunity against cancer. Several examples will be used to demonstrate these strategies. Using prodrugs, systemically administered drugs converted to toxic metabolites in the local tumor milieu, has proven to be a useful strategy for achieving high local concentrations of the toxic product while avoiding the systemic toxicity that limits the use of chemotherapy agents. Transfer of genes encoding cytosine deaminase (with 5-fluorocytosine) and carboxylesterase (CE) (with irinotecan) are two paradigms that have been used in our laboratory. The data demonstrate that using adenoviruses to deliver these genes to the tumor site leads to production of the active chemotherapeutic agent, which diffuses from the cell in which it was produced to suppress tumor growth and attain regional control in a single organ. Extensive experimental and clinical data now exist to support the concept that tumor growth is critically dependent on angiogenesis and that vascular endothelial growth factor (VEGF) appears to play a central role in the process of tumor neovascularization. Data generated in our laboratory have shown that adenovirus-mediated regional anti-VEGF therapy using a gene encoding a soluble form of flt-1 (one of the VEGF receptors) can be used for regional control of tumor growth. The critical dependence of many tumors on VEGF for neovascularization and dissemination predicts the general applicability of this strategy for treatment of many solid tumors. Another paradigm involves dendritic cells, potent antigen-presenting cells that play a critical role in the initiation of antitumor immune responses. Immunization of mice with dendritic cells genetically modified using an adenovirus vector transferring a gene encoding a tumor antigen confers potent protection against a lethal tumor challenge, as well as suppression of preestablished tumors, resulting in a significant survival advantage. One clinical scenario to which this approach is relevant is treating micrometastases present at the time of primary detection of many malignancies. A possible clinical strategy would be to modify dendritic cells from such patients using an adenovirus vector encoding the relevant tumor antigen, and then administering the genetically modified dendritic cells as adjuvant treatment following primary therapy.

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R.G. Crystal
Division of Pulmonary and Critical Care Medicine,
The New York Hospital – Cornell Medical Center,
New York, NY, USA

R.G. Crystal (✉)
520 East 70th Street, ST 505,
New York, NY 10021, USA
Tel.: +1 212 746-2258; Fax +1 212 746-8383
E-mail: geneticmedicine@mail.med.cornell.edu

Introduction

Gene therapy represents a treatment strategy where genetic information, usually in the form of DNA, is used to modify the genetic repertoire of patient target cells for therapeutic purposes [2, 15, 72, 104]. For any gene therapy application, the therapeutic goal dictates the choice of which gene is to be used, which strategy will be used to transfer the gene to the nucleus of the target cells, and whether the gene transfer process will be accomplished ex vivo or in vivo. Because the process of transferring genes is inefficient, it is usually accomplished by combining the gene with a vector, typically a modified virus or liposome. Ex vivo gene therapy describes a strategy where target cells are genetically

modified in the laboratory, and the altered cells are then administered to the individual. In contrast, in vivo gene therapy is a strategy where the genetic information is directly administered to the individual, and as such is analogous to the administration of conventional drugs.

In the past several years, the author and coworkers have been interested in the use of adenovirus (Ad) gene transfer vectors for the treatment of malignant disorders. To provide an overview of our work in this area, this review describes several strategies regarding cancer therapy using Ad vectors. In in vivo prodrug strategies, an Ad vector is used to deliver to the tumor a gene encoding an enzyme that converts an innocuous prodrug to an active chemotherapeutic agent in the local milieu. This provides a means of lowering the systemic dose of the chemotherapeutic agent. In an antiangiogenesis strategy, an Ad vector is used in vivo to deliver to the organ containing the tumor a soluble form of the flt-1 receptor for vascular endothelial growth factor (VEGF). This prevents tumors from using VEGF to stimulate local angiogenesis, thus limiting the ability of tumors to obtain an adequate blood supply. In an ex vivo strategy, an Ad vector is used to modify dendritic cells (DCs) genetically with a gene encoding a tumor antigen. The modified DCs are administered to the syngeneic host, thus providing a means for "professional" antigen-presenting cells to stimulate immunity against the tumor.

Ad gene transfer vectors

Ads are DNA viruses containing a 36-kDa double-stranded viral genome surrounded by a protein capsid [for a general review of Ads see 87; for reviews of Ad gene transfer vectors see 9, 20, 34, 104]. There are 49 Ad serotypes, categorized into 6 subgroups. The Ads conventionally used for gene transfer are all from subgroup C, usually serotype 5 or 2. The Ad genome comprises early (E1–E4) and late (L1–L5) genes. For most gene therapy applications, the E1 genes are deleted, rendering the virus unable to replicate. For cancer therapy applications, the E3 genes are usually removed to make room for the expression cassette, and to eliminate genes that Ads use to hide from antiviral host defenses. Other regions of the Ad genome, such as the E2 and E4 genes, can also be removed, although the advantages of doing this for tumor therapy applications are not proven.

The expression cassette, containing a promoter and the therapeutic gene, is usually inserted into the E1 position (Fig. 1). The infectious but replication-incompetent vector is produced by transfecting recombinant DNA into 293 human embryonic kidney cells, a cell line that provides the products of the missing Ad E1 genes, permitting the virus to replicate. Following purification by CsCl gradient centrifugation and dialysis, the Ad vector is stored at −70°C until use. With this approach, 10^{13} Ads with a titer of 10^{9}–10^{10} plaque-forming units (pfu)/mL can be easily produced.

Prodrug gene therapy

The concept underlying prodrug gene therapy is localization of chemotherapy to the region where the transgene has been transferred, thus concentrating high concentrations of the active chemotherapeutic agent at that site [14, 17, 82]. All gene therapy prodrug strategies are based on the theoretical concept that the systemic

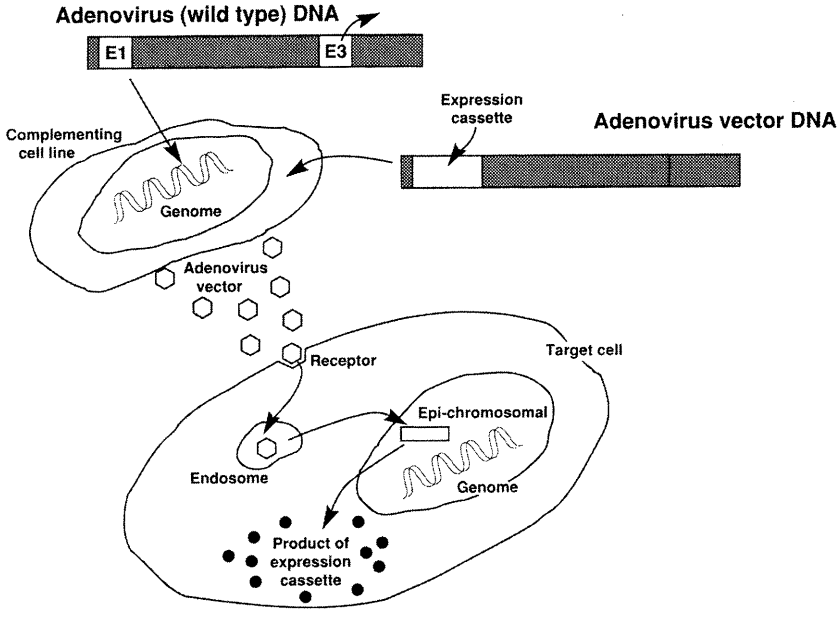

Fig. 1 Ad vector design, production, and gene transfer. To produce an Ad vector, the E1 sequences (and E3 sequences if the space is needed) are deleted. The expression cassette containing the promoter and transgene is inserted (usually in the E1 position) and the vector DNA is transfected into a complementary cell line with E1 sequences in its genome. The Ad vector with its expression cassette is $E1^{-}$ and thus incapable of replicating. The vector binds to the target cell through interactions of the Ad fiber and penton base with specific receptors, enters the cell via a cytoplasmic endosome, breaks out, and delivers its linear, dsDNA genome with the expression cassette into the nucleus where it functions in an epichromosomal fashion to direct the expression of its product. Reproduced, with permission, from Crystal et al. [15]

toxicity associated with chemotherapeutic agents might be minimized by transferring to tumor cells and the surrounding tissues an enzyme capable of converting an administered inactive prodrug to a toxic chemotherapeutic agent, thus achieving high concentrations of the toxic product only in the milieu of the tumor [33, 68]. Examples of this strategy include transfer of the herpes simplex virus thymidine kinase gene to allow cells to convert ganciclovir to active agents to suppress growth of tumors [13, 16, 19, 47, 60, 93, 98]; transfer of cytosine deaminase (CD) to convert 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU) for the treatment of colorectal cancer [38, 44, 70, 71, 76, 101]; transfer of deoxycytidine kinase to convert cytarabine to cytarabine 5′-phosphate for the treatment of glioma [62]; transfer of nitroreductase to activate CB1954 for the treatment of colorectal cancer [32]; and transfer of carboxypeptidase G2 to activate 4-[(2-chloroethyl) (2-mesyloxyethyl) amino] benzoyl-L-glutamic acid (CMDA) for the treatment of ovarian cancer [63].

5-FC/CD/5-FU

In vivo gene therapy with the CD gene is a paradigm of how to provide high local concentrations of 5-FU to colorectal carcinoma metastatic to the liver [3, 38, 42–44, 68, 70, 71, 81, 101]. CD catalyzes the deamination of cytosine to uracil, and thus the conversion of 5-FC to 5-FU [68]. Mammalian cells do not produce CD, but the enzyme is present in bacteria and fungi [1, 3, 42, 51, 70]. In contrast to the multiple adverse effects of systemic administration of 5-FU, systemic administration of 5-FC is remarkably free of complications [6]. In tumor models in experimental animals, when the cytosine deaminase gene is transferred to colon carcinoma cells and the animals are treated with systemic 5-FC, high local concentrations of 5-FU are produced by intratumoral conversion of 5-FC to 5-FU. This has been demonstrated in ex vivo models using the CD gene [42, 43, 71] and in an in vivo model of subcutaneous tumors using direct Ad-mediated delivery of the CD gene to tumor cells [38].

While direct in vivo transfer of the CD gene plus systemic 5-FC to well-defined colorectal carcinoma metastases is a useful demonstration of the ability of in vivo gene transfer to suppress tumor growth, in the clinical setting such a strategy will be limited to circumstances where there are a few, well-defined, detectable metastatic lesions. It would be more useful if this strategy could be applied to the more common clinical scenario of multiple hepatic metastases, particularly for suppressing the growth of undetected micrometastases. Thus for in vivo gene therapy with the CD gene plus systemic 5-FC to be broadly useful, it will be necessary to adapt this prodrug strategy to regional therapy. In this approach, the CD gene would be delivered to a significant proportion of the liver, allowing local conversion of the systemically administered 5-FC to provide high local concentrations of 5-FU. Theoretically, the 5-FU would diffuse regionally, suppressing the growth of the carcinoma while not causing significant toxicity to the normal liver.

Ohwada et al. [76] have developed a strategy directed toward this therapeutic goal, i.e., to use Ad-mediated regional transfer of the CD gene together with systemic administration of 5-FC to suppress the growth of small liver metastases of colon carcinoma cells. To accomplish this, an animal model of liver metastases of human colon carcinoma was established in nude mice, an Ad vector containing the CD gene (AdCD vector) was transferred to the lobe of the liver containing the metastases, and systemic 5-FC was administered to the animals. Based on the knowledge that there is a bystander effect when the AdCD vector is transferred to a limited number of cells in a population in the presence of 5-FC, it was hypothesized that the 5-FU (and its metabolites) produced by the local CD/5-FC combination would diffuse regionally and provide local concentrations of 5-FU sufficient to suppress the growth of colon carcinoma cells. The data demonstrate that suppression of tumor growth is feasible using regional therapy, with limited and transient toxicity to liver hepatocytes (Fig. 2A [76]). This approach expands the concept of Ad vector-mediated CD gene prodrug therapy of colorectal carcinoma

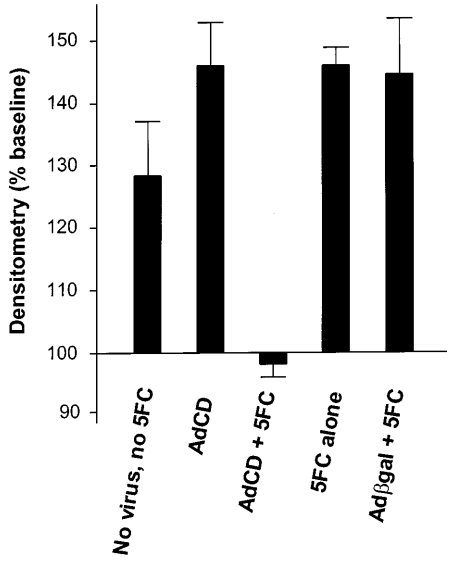

Fig. 2 Suppression of the growth of tumors of human colon carcinoma cells in nude mouse liver by an Ad vector expressing the *E. coli* cytosine deaminase gene (AdCD) with concomitant administration of the prodrug 5-FC. The data represent the densitometric values of dot-blot analyses of human DNA (tumor DNA) in mouse liver presented as a percentage of baseline values (amount of human DNA in the mouse liver at day 0, before therapy). The data includes densitometric values obtained 21 days after therapy from uninfected animals not treated with 5-FC (no virus, no 5-FC); AdCD-infected animals not treated with 5-FC (AdCD alone); AdCD-infected animals treated with 5-FC (AdCD + 5-FC); uninfected animals treated with 5-FC (5-FC alone); and Adβgal-infected (control Ad expressing the β-gal gene) animals treated with 5-FC (Adβgal + 5-FC). Reproduced, with permission, from Ohwada et al. [76]

metastases to the liver from direct administration [38] to regional control of the growth of micrometastases. We have observed similar results when the AdCD vector has been administered to the liver by the intravenous route [101].

Irinotecan/CE/SN-38

The irinotecan/CE/SN-38 strategy, developed by Kojima et al. [54], uses a cDNA encoding a human enzyme (CE) to concentrate an active chemotherapeutic agent at the site of a tumor. Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin), a camptothecin analogue that functions as an inhibitor of mammalian DNA topoisomerase I, is a chemotherapeutic agent that is active against lung, cervical, ovarian, and colorectal tumors, as well as leukemias and lymphomas [29, 64, 75, 90, 97]. The drug is unique among the camptothecins in that it has a bulky piperidino side chain at the C-10 position [56]. This side chain can be cleaved enzymatically by CE to produce SN-38 (7-ethyl-10-hydroxycamptothecin), which functions as a topoisomerase I inhibitor that is 1000-fold more potent than irinotecan [48]. Since CE activity is found in serum and some internal organs, irinotecan is classified as a prodrug, being activated in vivo to produce the more potent SN-38 [46]. In contrast to irinotecan, which is soluble in aqueous solutions, SN-38 is insoluble, and thus not useful as a chemotherapeutic agent despite its potency [58]. Although irinotecan is now available as a chemotherapeutic agent for some indications, its usefulness is limited by dose-dependent toxicity, primarily diarrhea and granulocytopenia [73].

The strategy developed by Kojima et al. [54] circumvents the toxicity of irinotecan by transferring the CE gene directly to the tumor, then administering irinotecan directly to the tumor, i.e., a regional chemotherapeutic strategy in which the prodrug irinotecan would be converted to the more active SN-38 within the tumor, consequently maximizing the exposure of the tumor cells to the active agent while limiting systemic concentrations of the drug and thus the systemic toxicity associated with conventional use of irinotecan. To evaluate this strategy, Kojima et al. constructed a recombinant, replication-deficient Ad vector containing human CE cDNA and used the combination of local delivery of this gene transfer vector and CPT-11 to suppress the growth of lung tumor cells [54]. The data demonstrated that by using an Ad vector to transfer CE cDNA, irinotecan was incorporated by the tumor and converted to SN-38, with consequent antitumor effects (Fig. 3).

One theoretical advantage of the AdCE/irinotecan strategy is a "bystander" effect, where the active chemotherapeutic agent diffuses from the tumor cell in which it was produced to neighboring malignant cells in sufficient concentrations to suppress growth. In this regard, infection with the AdCE vector and the addition of irinotecan alters the sensitivity of tumor cells in vitro to allow toxicity at 7- to 17-fold lower doses of irinotecan, with as few as 10% of A549 cells needing to be infected with AdCE to result in cytotoxicity for as much as 48% of the total population of tumor cells [54]. In the context that in vivo infection with gene transfer vectors has effectiveness limited to the proportion of the total number of tumor cells that is infected [38, 44, 101], this approach serves as a model for local adjuvant therapy for solid tumors, such as the treatment of intrabronchial tumors, tumors invading the chest wall, and inoperable intrapulmonary tumors.

Antiangiogenesis

The growth of tumors greater than 1–2 mm^3 in volume is critically dependent on angiogenesis to provide nutrients to the growing tumor [23, 27]. Among the several mediators produced by tumor cells that have been implicated in tumor angiogenesis [23, 26, 27, 53, 69], VEGF appears to play a central role (Fig. 4A) [23, 25, 26, 52, 53, 69, 86, 88]. In this regard, VEGF is overexpressed in most human tumors and tumor cell lines [69, 85, 88, 91, 92]. The single-copy VEGF gene encodes a dimeric glycoprotein that is secreted from cells in five isoforms, of which the 121- and 165-amino acid forms dominate in tumors [21, 59, 78, 84, 100]. The VEGF dimer signals endothelial cells to proliferate, migrate, and form capillary tubes via interactions with flt-1 and/or flk-1/KDR, high-affinity receptors found almost exclusively on endothelial cells [5, 18, 21, 36, 49, 65, 66, 79, 89, 99, 100]. flt-1 and flk-1/KDR are membrane-spanning receptors characterized by the presence of seven immunoglobulin-like sequences in their extracellular domain, a transmembrane domain, and intracellular tyrosine kinase domains containing a kinase insert sequence [5, 18, 21, 36, 49, 65, 66, 79, 89, 99, 100]. In response to binding of the VEGF dimer, dimerization of the membrane-bound receptors occurs, followed by activation of the intracellular tyrosine kinase domain, autophosphorylation of the receptors, and consequent tyrosine phosphorylation of mediators of intracellular signal transduction [36, 100].

Since VEGF is overexpressed in most tumors, one strategy to block the neovascularization of tumors is to infuse the tumor with a truncated, soluble form of a VEGF receptor that will sequester VEGF produced by the tumor and/or interact with the VEGF receptors in the local milieu in a dominant-negative fashion, blocking their ability to be triggered by VEGF [49, 50, 55, 67, 100]. One challenge for this approach to tumor therapy is how to achieve high concentrations of the truncated receptor within the organ containing the tumor without the potential risk of generalized inhibition of angiogenesis in other organs which normally require basal, physiologic levels of neovascularization [4, 10, 11, 28, 35, 80, 102].

The strategy developed by Kong et al. [55] hypothesized that regional anti-VEGF therapy, and hence re-

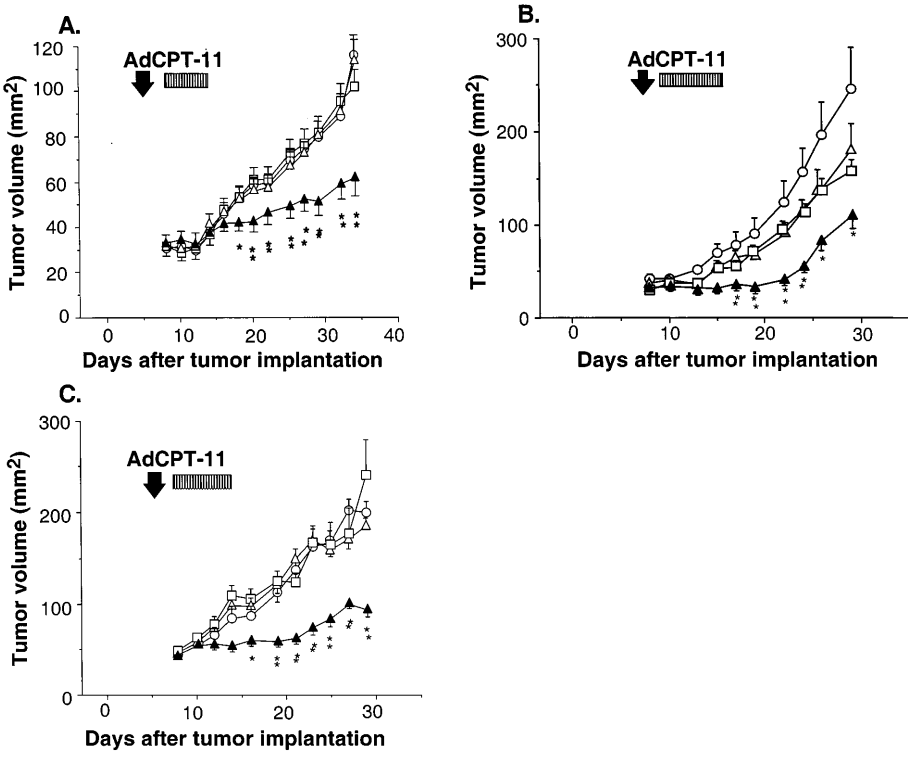

Fig. 3A–C In vivo growth of subcutaneous (**A**), A549 human lung adenocarcinoma cell (**B**) H157 human lung adenocarcinoma cell, and (**C**) HeLa cervical carcinoma cell tumors following local administration of an Ad vector expressing the human CE gene (AdCE) with concomitant administration of the prodrug irinotecan. Tumors were established in Balb/c nu/nu mice by subcutaneous injection of 8×10^6 cells. After 7 days, tumors were randomized to receive AdCE 10^9 pfu followed by irinotecan (▲) or phosphate-buffered saline (PBS; □); AdNull10^9 pfu (a control Ad without a transgene) followed by irinotecan (△); or PBS only (○). Ad or PBS was delivered intratumorally in a volume of 100 μL on day 8. After 24 h, the animals received once-daily intratumoral injections of 100 μL of irinotecan 10 μg/mL or PBS until day 15. The data are expressed as means ± standard error of the mean. $*P < 0.05$, †$P < 0.01$ for size of tumors in mice treated with AdCE followed by CPT-11 compared with other groups. Reproduced, with permission, from Kojima et al. [54]

gional control of tumor growth, might be achieved by Ad-mediated regional transfer of the cDNA that encodes a secreted form of the extracellular domain of the human flt-1 VEGF receptor (Ad*sflt*). Using subcutaneous, liver metastatic, and lung metastatic models of tumor growth, the data demonstrated that the regional delivery of the *sflt* cDNA via an Ad vector dramatically inhibits the growth of preestablished tumors in animal models, but only within the organs to which the vector is administered, i.e., the Ad*sflt* vector functions in a regional fashion, providing anti-VEGF therapy only in the milieu to which the therapy is directed (Fig. 4B, C) [55]. Of major importance is the finding that the effects are regional, i.e., the suppression of tumor growth is confined to the target organs to which the Ad vector is delivered. Thus by administering *sflt* via gene transfer with an Ad vector, striking regional suppression of tumor growth is achieved, thus obviating the theoretical risk of promiscuous systemic antiangiogenesis.

These observations are also important in regard to the general concept of the process of tumor-induced neovascularization, conceptualized as an imbalance between angiogenic stimulators and inhibitors in the milieu of the tumor in favor of the stimulators [23, 24, 26, 39, 61, 74]. Although it is recognized that several angiogenic mediators are involved in tumor angiogenesis, the finding that regional disruption of the interaction between VEGF and its native receptors leads to significant tumor suppression underscores the pivotal role of the VEGF/VEGF receptor system in tumor angiogenesis [52, 69, 85, 86, 88]. In this regard, the critical dependence of many tumors on VEGF for neovascularization for their growth and dissemination predicts the general applicability of the soluble VEGF receptor strategy to the treatment of many solid tumors [55].

Genetically modified DCs

Many tumor cells express epitopes on their surface that provide a potential target for therapeutic vaccine strategies that boost natural immune-mediated tumor defense mechanisms [8, 40, 77, 94]. One challenge in developing antitumor vaccines is to focus the immune response specifically on the relevant tumor antigen. Song et al. [94] approached this challenge by using replication-deficient, recombinant Ad vectors to transfer a gene encoding a tumor antigen to DCs ex vivo and administering the genetically modified DC to a syngeneic host. This approach was chosen to capitalize on the

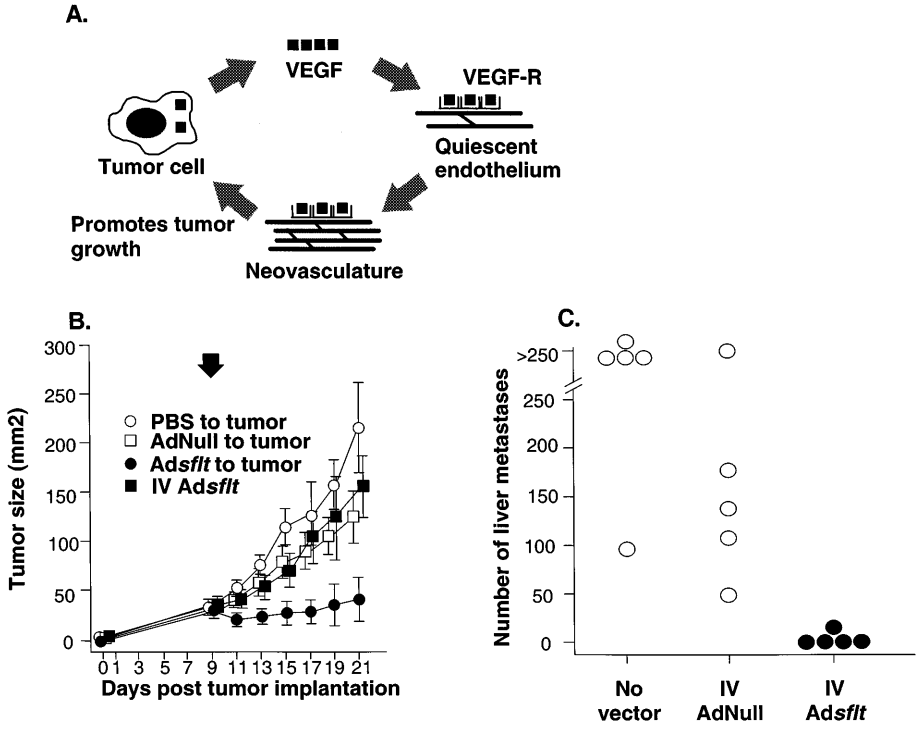

Fig. 4A–C Antiangiogenesis mediated by Ad vector-mediated delivery of cDNA encoding the extracellular, soluble form of the flt-1 receptor (sflt-1) for VEGF. (**A**) Concept of "autoangiogenesis" mediated by tumor cells. The tumor cells produce VEGF which interacts with VEGF receptors (VEGF-Rs) on quiescent endothelium in the local milieu. This induces neovasculaturization which, in turn, helps the tumor grow. By providing a soluble form of flt-1 using an Ad vector, the VEGF–VEGF-R interaction is interrupted, thus preventing the tumor from inducing the neovasculaturization necessary for tumor growth. (**B**) and (**C**) Ad*sflt* expresses a naturally occurring soluble, secreted form of the human flt-1 receptor. The product is identical to amino acids 1 through 657 of the full-length receptor with a 31-amino acid C-terminal extension. The cDNA encodes six of the seven extracellular immunoglobulin-like domains, but lacks the transmembrane and intracellular domains. AdNull has no transgene and was used as a control. (**B**) Effect of regional vs systemic in vivo administration of Ad*sflt* on the growth of primary subcutaneous tumors. CT26.CL25 colon carcinoma cells modified to express β-gal [106] were allografted subcutaneously into syngeneic Balb/c mice. Nine days later, PBS (○), AdNull 5×10^8 pfu (□), or Ad*sflt* (●) was administered directly into tumors that measured 25–30 mm^2 or Ad*sflt* 5×10^8 pfu was administered intravenously (■). Data are means ± standard error. (**C**) Quantification of the number of liver metastases in untreated, AdNull-treated, and Ad*sflt*-treated animals. To evaluate the ability of the Ad*sflt* vector to suppress preestablished metastatic liver tumors, 3×10^5 CT26.CL25 cells were injected through the lower pole of the exteriorized spleen to establish a primary splenic tumor and multiple hematogenously disseminated liver metastases. Three days following tumor implantation, Ad*sflt* 5×10^8 pfu or AdNull 5×10^8 pfu was administered intravenously through the jugular vein, a route by which tumor cells are known to be distributed to the liver (90% of a dose goes to the liver and approximately 5% to the spleen [37, 41, 105]). Animals were killed 15 days following vector administration, and livers were harvested and stained for β-gal expression. To quantify the tumor burden, the number of liver metastases were counted in a blinded fashion with the aid of a dissecting microscope. Each data point represents an individual animal. Only ≤250 metastatic deposits per liver could be reliably enumerated; livers with >250 deposits were assigned an empirical number of 250 [57]. Reproduced, with permission, from Kong et al. [55]

biologic properties of Ad vectors and DCs that are relevant to inducing antigen-specific cytotoxic T lymphocytes (CTLs) directed against specific antigens. In this context, there is emerging evidence that in some circumstances Ad vectors can act as adjuvants to boost the CTL response against heterologous transgenes [12, 22, 45, 83, 94, 106]. This evidence, together with the knowledge that genes expressed by Ad vectors are expressed through the class I pathway [7, 30] and that DCs are the most potent members of the general class of antigen-presenting cells [31, 95, 96, 107], led us to the hypothesis that by genetically modifying DCs using an Ad vector encoding a tumor antigen might help focus the immune response on generating antigen-specific CTLs.

To assess the ability of Ad vectors to modify DCs genetically ex vivo and the ability of DCs modified with model antigens and transferred to a syngeneic host to induce antigen-specific CTLs, prevent tumors from developing in animals challenged with tumor cells expressing the model antigen, and suppress the growth of preexisting tumors composed of the same tumor cells, we utilized freshly isolated DCs from Balb/c mouse bone marrow. By using an Ad vector expressing β-galactosidase (β-gal) as a model tumor antigen and the Balb/c syngeneic colon carcinoma cell line CT26.CL25 expressing β-gal [103], the data demonstrated that administration of DCs modified to express β-gal induces β-gal-specific CTLs, prevents tumor growth, and suppresses the growth of preexisting tumors (Fig. 5).

In vivo administration of these genetically modified DCs elicits a major histocompatibility complex-restricted, antigen-specific CTL response, protects syngeneic mice against tumor challenge, and suppresses preexisting lethal tumors. Importantly, a reduction in tumor burden

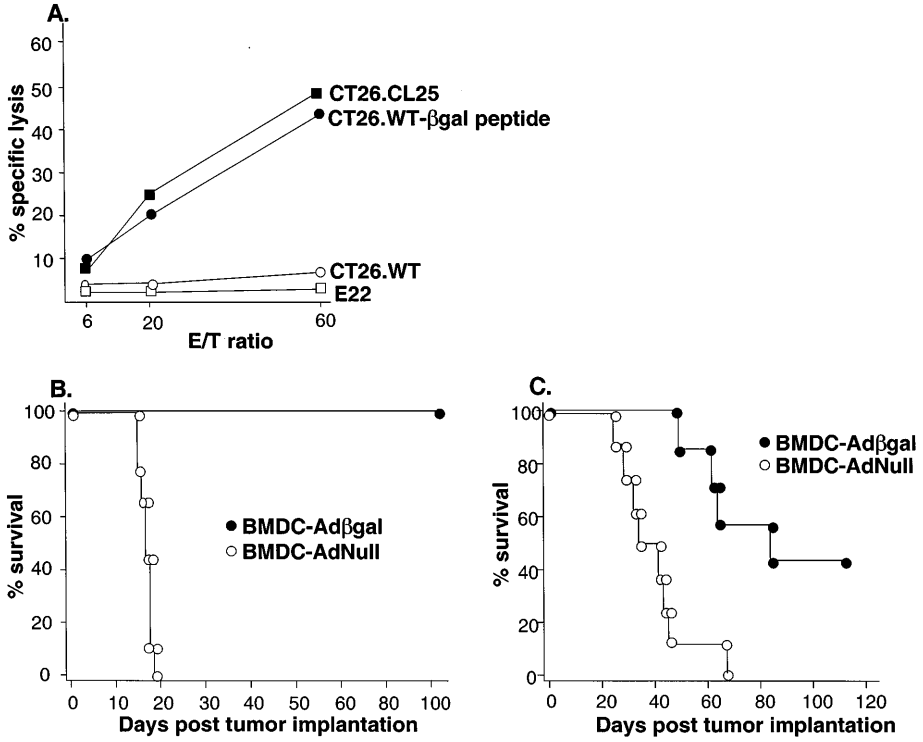

Fig. 5A–C Demonstration of the ability of DCs genetically modified to express β-gal, a model tumor antigen, to induce specific CTLs directed against β-gal, to protect syngeneic mice against challenge with a tumor expressing β-gal, and to suppress the growth of tumors expressing β-gal. (**A**) Induction of CTL response in Balb/c mice following in vivo administration of bone marrow-derived DCs (BMDCs) infected with Adβ-gal, an Ad vector expressing β-gal. BMDCs were transduced in vitro with Adβ-gal (multiplicity of infection 100, 2 h). Twenty-four hours later, 3×10^5 CT26.CL25 cells (Balb/c syngeneic colon carcinoma cells modified to express β-gal) were administered subcutaneously into Balb/c mice. Fourteen days later, splenocytes harvested from immunized mice were stimulated for 5 days in vitro with syngeneic fibroblasts pulsed with β-gal peptide and then assayed for specific cell lysis of three syngeneic colon carcinoma target cell types (parental CT26.WT cells, which are identical to CT26.CL25, but do not express β-gal [●]; β-gal-expressing CT26.CL25 cells [■]; and CT26.WT cells pulsed with β-gal peptide in vitro [○]) as well as the E22 β-gal-expressing allogeneic tumor cell line (□). (**B**) Survival advantage in mice immunized with Adβ-gal-modified BMDCs following a tumor challenge with CT26.CL25 cells. Animals were immunized with BMDCs modified with Adβ-gal (●) or AdNull (○), followed 14 days later by intravenous challenge with 10^5 CT26.CL25 tumor cells. The animals were not killed but followed for survival. Data are expressed as % survival as a function of time. Survival of mice immunized with BMDC-Adβ-gal was significantly greater than that of BMDC-AdNull control mice, as determined by log-rank analysis of the Kaplan-Meier survival curves ($P < 0.0001$). (**C**) Survival advantage in CT26.CL25 tumor-bearing mice treated with Adβ-gal-modified BMDCs. Three days following the establishment of lung metastases by intravenous administration of 3×10^4 CT26.CL25 tumor cells, Balb/c mice were immunized with BMDCs modified with Adβ-gal or AdNull. The animals were followed for survival. Data are expressed as % survival as a function of time. Survival of mice treated with BMDC-Adβ-gal was significantly greater than that of BMDC-AdNull control mice as determined by log-rank analysis of the Kaplan-Meier survival curves ($P < 0.002$). Reproduced, with permission, from Song et al. [94]

translated into a significant survival advantage [94]. These observations underscore the ability of genetically modified DCs to present tumor antigens and induce immunity against tumors effectively. With the discovery and definition of more tumor antigens, this strategy of genetic manipulation of DCs to express specific tumor antigens may provide an effective treatment for an increasing number of malignancies.

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References

1. Andersen L, Kilstrup M, Neuhard J (1989) Pyrimidine, purine and nitrogen control of cytosine deaminase synthesis in *Escherichia coli* K 12. Involvement of the glnLG and purR genes in the regulation of codA expression. Arch Microbiol 152: 115
2. Anderson WF (1998) Human gene therapy. Nature 392: 25
3. Austin EA, Huber BE (1993) A first step in the development of gene therapy for colorectal carcinoma: cloning, sequencing, and expression of *Escherichia coli* cytosine deaminase. Mol Pharmacol 43: 380
4. Banai S, Shweiki D, Pinson A, Chandra M, Lazarovici G, Keshet E (1994) Upregulation of vascular endothelial growth factor expression induced by myocardial ischaemia: implications for coronary angiogenesis. Cardiovasc Res 28: 1176
5. Barleon B, Hauser S, Schollmann C, Weindel K, Marme D, Yayon A, Weich HA (1994) Differential expression of the two VEGF receptors flt and KDR in placenta and vascular endothelial cells. J Cell Biochem 54: 56
6. Bennett J (1998) Antifungal agents. In: Gilman AG, Rall T, Nies AS, Taylor P (eds), Goodman and Gilman's the pharmacological basis of therapeutics. Pergamon Press, New York, p. 1165

7. Bevan MJ (1995) Antigen presentation to cytotoxic T lymphocytes in vivo. J Exp Med 182: 639
8. Boon T, van der Bruggen P (1996) Human tumor antigens recognized by T lymphocytes. J Exp Med 183: 725
9. Bramson JL, Graham FL, Gauldie J (1995) The use of adenoviral vectors for gene therapy and gene transfer in vivo. Curr Opin Biotechnol 6: 590
10. Brogi E, Schatteman G, Wu T, Kim EA, Varticovski L, Keyt B, Isner JM (1996) Hypoxia-induced paracrine regulation of vascular endothelial growth factor receptor expression. J Clin Invest 97: 469
11. Brown LF, Yeo KT, Berse B, Yeo TK, Senger DR, Dvorak HF, Van de Water L (1992) Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. J Exp Med 176: 1375
12. Chen PW, Wang M, Bronte V, Zhai Y, Rosenberg SA, Restifo NP (1996) Therapeutic antitumor response after immunization with a recombinant adenovirus encoding a model tumor-associated antigen. J Immunol 156: 224
13. Chen SH, Shine HD, Goodman JC, Grossman RG, Woo SLC (1994) Gene therapy for brain tumors: regression of experimental gliomas by adenovirus-mediated gene transfer in vivo. Proc Natl Acad Sci USA 91: 3054
14. Connors TA (1995) The choice of prodrugs for gene directed enzyme prodrug therapy of cancer. Gene Ther 2: 702
15. Crystal RG (1995) Transfer of genes to humans: early lessons and obstacles to success. Science 270: 404
16. Culver KW, Ram Z, Wallbridge S, Ishii H, Oldfield EH, Blaese RM (1992) In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. Science 256: 1550
17. Davis BM, Koc ON, Lee K, Gerson SL (1996) Current progress in the gene therapy of cancer. Curr Opin Oncol 8: 499
18. de Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT (1992) The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 255: 989
19. Di Maio JM, Clary BM, Via DF, Coveney E, Pappas TN, Lyerly HK (1994) Directed enzyme pro-drug gene therapy for pancreatic cancer in vivo. Surgery 116: 205
20. Falck-Pedersen E (1998) Use and application of adenovirus expression vectors. In: Spector DL, Goldman RD, Leinwand LA (eds), Cells: a laboratory manual. Cold Spring Harbor Laboratory Press, Plainview, NY
21. Ferrara N, Houck K, Jakeman L, Leung DW (1992) Molecular and biological properties of the vascular endothelial growth factor family of proteins. Endocr Rev 13: 18
22. Flanagan B, Pringle CR, Leppard KN (1997) A recombinant adenovirus expressing the simian immunodeficiency virus Gag antigen can induce long-lived immune responses in mice. J Gen Virol 78: 991
23. Folkman J (1995) Tumor angiogenesis. In: Mendelsoh J, Howley PM, Israel MA, Liotta LA (eds), The molecular basis of cancer. W.B. Saunders, Philadelphia, p. 206
24. Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. Nature Med 1: 27
25. Folkman J, Klagsburn M (1987) Angiogenic factors. Science 235: 442
26. Folkman J, Shing Y (1992) Angiogenesis. J Biol Chem 267: 10931
27. Folkman J, Cole P, Zimmerman S (1966) Tumor behavior in isolated perfused organs: in vitro growth and metastases of biopsy material in rabbit thyroid and canine intestinal segment. Ann Surg 164: 491
28. Frank S, Hubner G, Breier G, Longaker MT, Greenhalgh DG, Werner S (1995) Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. J Biol Chem 270: 12607
29. Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, Nishiwaki Y, Kuriyama T, Ariyoshi Y, Negoro S, Masuda N, Nakajima S, Taguchi T (1992) A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. J Clin Oncol 10: 16
30. Germain RN (1994) MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell 76: 287
31. Grabbe S, Beissert S, Schwarz T, Granstein RD (1995) Dendritic cells as initiators of tumor immune responses: a possible strategy for tumor immunotherapy? Immunol Today 16: 117
32. Green NK, Youngs DJ, Neoptolemos JP, Friedlos F, Knox RJ, Springer CJ, Anlezark GM, Michael NP, Melton RG, Ford MJ, Young LS, Kerr DJ, Searle PF (1997) Sensitization of colorectal and pancreatic cancer cell lines to the prodrug 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB1954) by retroviral transduction and expression of the *E. coli* nitroreductase gene. Cancer Gene Ther 4: 229
33. Gutierrez AA, Lemoine NR, Sikora K (1992) Gene therapy for cancer. Lancet 339: 715
34. Haddada H, Cordier L, Perricaudet M (1995) Gene therapy using adenovirus vectors. Curr Top Microbiol Immunol 199: 297
35. Hang J, Kong L, Gu JW, Adair TH (1995) VEGF gene expression is upregulated in electrically stimulated rat skeletal muscle. Am J Physiol 269: H1827
36. Heldin CH (1995) Dimerization of cell surface receptors in signal transduction. Cell 80: 213
37. Herz J, Gerard RD (1993) Adenovirus-mediated transfer of low density lipoprotein receptor gene acutely accelerates cholesterol in normal mice. Proc Natl Acad Sci USA 90: 2812
38. Hirschowitz EA, Ohwada A, Pascal WR, Russi TJ, Crystal RG (1995) In vivo adenovirus-mediated gene transfer of the *Escherichia coli* cytosine deaminase gene to human colon carcinoma-derived tumors induces chemosensitivity to 5-fluorocytosine. Hum Gene Ther 6: 1055
39. Holmgren L, O'Reilly MS, Folkman J (1995) Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nature Med 1: 149
40. Houghton AN (1994) Cancer antigens: immune recognition of self and altered self. J Exp Med 180: 1
41. Huard J, Lochmuller H, Acsadi G, Jani A, Massie B, Karpati G (1995) The route of administration is a major determinant of the transduction efficiency of rat tissues by adenoviral recombinants. Gene Ther 2: 107
42. Huber BE, Richards CA, Krenitsky TA (1991) Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: an innovative approach for cancer therapy. Proc Natl Acad Sci USA 88: 8039
43. Huber BE, Austin EA, Good SS, Knick VC, Tibbels S, Richards CA (1993) In vivo antitumor activity of 5-fluorocytosine on human colorectal carcinoma cells genetically modified to express cytosine deaminase. Cancer Res 53: 4619
44. Huber BE, Austin EA, Richards CA, Davis ST, Good SS (1994) Metabolism of 5-fluorocytosine to 5-fluorouracil in human colorectal tumor cells transduced with the cytosine deaminase gene: significant antitumor effects when only a small percentage of tumor cells express cytosine deaminase. Proc Natl Acad Sci USA 91: 8302
45. Juillard V, Villefroy P, Godfrin D, Pavirani A, Venet A, Guillet JG (1995) Long-term humoral and cellular immunity induced by a single immunization with replication-defective adenovirus recombinant vector. Eur J Immunol 25: 3467
46. Kaneda N, Nagata H, Furuta T, Yokokura T (1990) Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. Cancer Res 50: 1715
47. Kaneko S, Hallenbeck P, Kotani T, Nakabayashi H, McGarrity G, Tamaoki T, Anderson WF, Chiang YL (1995) Adenovirus-mediated gene therapy of hepatocellular carcinoma using cancer-specific gene expression. Cancer Res 55: 5283
48. Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K (1991) Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. Cancer Res 51: 4187

49. Kendall RL, Thomas KA (1993) Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci USA 90: 10705
50. Kendall RL, Wang G, Thomas KA (1996) Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. Biochem Biophys Res Commun 226: 324
51. Kilstrup M, Meng LM, Neuhard J, Nygaard P (1989) Genetic evidence for a repressor of synthesis of cytosine deaminase and purine biosynthesis enzymes in *Escherichia coli*. J Bacteriol 171: 2124
52. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N (1993) Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 362: 841
53. Klagsbrun M, D'Amore PA (1991) Regulators of angiogenesis. Annu Rev Physiol 53: 217
54. Kojima A, Hackett NR, Ohwada A, Crystal RG (1998) In vivo human CE cDNA gene transfer to activate the prodrug CPT-11 for local treatment of solid tumors. J Clin Invest 101: 1789
55. Kong HL, Hecht D, Song W, Kovesdi I, Hackett NR, Yayon A, Crystal RG (1998) Regional suppression of tumor growth by in vivo transfer of a cDNA encoding a secreted form of the extracellular domain of the flt-1 vascular endothelial growth factor receptor. Hum Gene Ther 9: 823
56. Kunimoto T, Nitta K, Tanaka T, Uehara N, Baba H, Takeuchi M, Yokokura T, Sawada S, Miyasaka T, Mutai M (1987) Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. Cancer Res 47: 5944
57. Lafreniere R, Rosenberg SA (1986) A novel approach to the generation and identification of experimental hepatic metastases in a murine model. J Natl Cancer Inst 76: 309
58. Lavelle F, Bissery MC, Andre S, Roquet F, Riou JF (1996) Preclinical evaluation of CPT-11 and its active metabolite SN-38. Semin Oncol 23: 11
59. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N (1989) Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246: 1306
60. Link CJ Jr, Kolb EM, Muldoon RR (1995) Preliminary in vitro efficacy and toxicities studies of the herpes simplex thymidine kinase gene system for the treatment of breast cancer. Hybridoma 14: 143
61. Liotta LA, Steeg PS, Stetler-Stevenson WG (1991) Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 64: 327
62. Manome Y, Wen PY, Dong Y, Tanaka T, Mitchell BS, Kufe DW, Fine HA (1996) Viral vector transduction of the human deoxycytidine kinase cDNA sensitizes glioma cells to the cytotoxic effects of cytosine arabinoside in vitro and in vivo. Nature Med 2: 567
63. Marais R, Spooner RA, Light Y, Martin J, Springer CJ (1996) Gene-directed enzyme prodrug therapy with a mustard prodrug/carboxypeptidase g2 combination. Cancer Res 56: 4735
64. Masuda N, Fukuoka M, Kusunoki Y, Matsui K, Takifuji N, Kudoh S, Negoro S, Nishioka M, Nakagawa K, Takada M (1992) CPT-11: A new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 10: 1225
65. Matthews W, Jordan CT, Gavin M, Jenkins NA, Copeland NG, Lemischka IR (1991) A receptor tyrosine kinase cDNA isolated from a population of enriched primitive hematopoietic cells and exhibiting close genetic linkage to c-kit. Proc Natl Acad Sci USA 88: 9026
66. Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller NP, Risau W, Ullrich A (1993) High affinity VEGF binding and developmental expression suggest flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 72: 835
67. Millauer B, Shawver LK, Plate KH, Risau W, Ullrich A (1994) Glioblastoma growth inhibited in vivo by a dominant-negative flk-1 mutant. Nature 367: 576
68. Moolten FL (1994) Drug sensitivity ("suicide") genes for selective cancer chemotherapy. Cancer Gene Ther 1: 279
69. Morrison RS, Yahanda AM (1995) Tumor angiogenesis and the role of paracrine mediators. In: Freireich EJ, Stass SA (eds), Molecular basis of oncology. Blackwell Science, Cambridge, MA, p. 163
70. Mullen CA, Kilstrup M, Blaese RM (1992) Transfer of the bacterial gene for cytosine deaminase to mammalian cells confers lethal sensitivity to 5-fluorocytosine: a negative selection system. Proc Natl Acad Sci USA 89: 33
71. Mullen CA, Coale MM, Lowe R, Blaese RM (1994) Tumors expressing the cytosine deaminase suicide gene can be eliminated in vivo with 5-fluorocytosine and induce protective immunity to wild type tumor. Cancer Res 54: 1503
72. Mulligan RC (1993) The basic science of gene therapy. Science 260: 926
73. Negoro S, Fukuoka M, Masuda N, Takada M, Kusunoki Y, Matsui K, Takifuji N, Kudoh S, Niitani H, Taguchi T (1991) Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small-cell lung cancer. J Natl Cancer Inst 83: 1164
74. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J (1994) Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 79: 315
75. Ohno R, Okada K, Masaoka T, Kuramoto A, Arima T, Yoshida Y, Ariyoshi H, Ichimaru M, Sakai Y, Oguro M, Ito Y, Morishima Y, Yokomaku S, Ota K (1990) An early phase II study of CPT-11: A new derivative of camptothecin, for the treatment of leukemia and lymphoma. J Clin Oncol 8: 1907
76. Ohwada A, Hirschowitz EA, Crystal RG (1996) Regional delivery of an adenovirus vector containing the *Escherichia coli* cytosine deaminase gene to provide local activation of 5-fluorocytosine to suppress the growth of colon carcinoma metastatic to liver. Hum Gene Ther 7: 1567
77. Pardoll DM (1993) Cancer vaccines. Immunol Today 14: 310
78. Poltorak Z, Cohen T, Sivan R, Kandelis Y, Spira G, Vlodavsky I, Keshet E, Neufeld G (1997) VEGF145, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix. J Biol Chem 272: 7151
79. Quinn TP, Peters KG, de Vries C, Ferrara N, Williams LT (1993) Fetal liver kinase 1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. Proc Natl Acad Sci USA 90: 7533
80. Reynolds LP, Killilea SD, Redmer DA (1992) Angiogenesis in the female reproductive system. FASEB J 6: 886
81. Richards CA, Austin EA, Huber BE (1995) Transcriptional regulatory sequences of carcinoembryonic antigen: identification and use with cytosine deaminase for tumor-specific gene therapy. Hum Gene Ther 6: 881
82. Rigg A, Sikora K (1997) Genetic prodrug activation therapy. Mol Med Today 3: 359
83. Rodriques EG, Zavala F, Eichinger D, Wilson JM, Tsuji M (1997) Single immunizing dose of recombinant adenovirus efficiently induces CD8 + T cell-mediated protective immunity against malaria. J Immunol 158: 1268
84. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF (1983) Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219: 983
85. Senger DR, Perruzzi CA, Feder J, Dvorak HF (1986) A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. Cancer Res 46: 5629
86. Senger DR, Van de Water L, Brown LF, Nagy JA, Yeo KT, Yeo TK, Berse B, Jackman RW, Dvorak AM, Dvorak HF (1993) Vascular permeability factor (VPF, VEGF) in tumor biology. Cancer Metastasis Rev 12: 303
87. Shenk T (1996) Adenoviridae: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM (eds), Fields virology. Lippincott-Raven Publishers, Inc. Philadelphia, p. 2111

88. Shibuya M (1995) Role of VEGF-flt receptor system in normal and tumor angiogenesis. Adv Cancer Res 67: 281
89. Shibuya M, Yamaguchi S, Yamane A, Ikeda T, Tojo A, Matsushime H, Sato M (1990) Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. Oncogene 5: 519
90. Shimada Y, Yoshino M, Wakui A, Nakao I, Futatsuki K, Sakata Y, Kambe M, Taguchi T, Ogawa N, The CPT-11 Gastrointestinal Cancer Study Group (1993) Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. J Clin Oncol 11: 909
91. Shweiki D, Itin A, Soffer D, Keshet E (1992) Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359: 843
92. Shweiki D, Neeman M, Itin A, Keshet E (1995) Induction of vascular endothelial growth factor expression by hypoxia and by glucose deficiency in multicell spheroids: implications for tumor angiogenesis. Proc Natl Acad Sci USA 92: 768
93. Smythe WR, Hwang HC, Amin KM, Eck SL, Davidson BL, Wilson JM, Kaiser LR, Albelda SM (1994) Use of recombinant adenovirus to transfer the herpes simplex virus thymidine kinase (HSVtk) gene to thoracic neoplasms: an effective in vitro drug sensitization system. Cancer Res 54: 2055
94. Song W, Kong HL, Carpenter H, Torii H, Granstein R, Rafii S, Moore MA, Crystal RG (1997) Dendritic cells genetically modified with an adenovirus vector encoding the cDNA for a model antigen induce protective and therapeutic antitumor immunity. J Exp Med 186: 1247
95. Steinman RM (1991) The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 9: 271
96. Steinman RM (1997) Dendritic cells and immune-based therapies. Exp Hematol 24: 859
97. Takeuchi S, Takamizawa H, Takeda Y, Ohkawa T, Tamaya T, Noda K, Sugawa T, Sekiba K, Yakushiji M, Taguchi T (1991) An early phase II study of CPT-11 in gynecologic cancers. Research Group of CPT-11 in Gynecologic Cancers. Gan To Kagaku Ryoho 18: 579 (In Japanese)
98. Tanaka T, Kanai F, Okabe S, Yoshida Y, Wakimoto H, Hamada H, Shiratori Y, Lan K, Ishitobi M, Omata M (1996) Adenovirus-mediated prodrug gene therapy for carcinoembryonic antigen-producing human gastric carcinoma cells in vitro. Cancer Res 56: 1341
99. Terman BI, Dougher-Vermazen M, Carrion ME, Dimitrov D, Armellino DC, Gospodarowicz D, Bohlen P (1992) Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun 187: 1579
100. Thomas KA (1996) Vascular endothelial growth factor, a potent and selective angiogenic agent. J Biol Chem 271: 603
101. Topf N, Worgall S, Hackett NR, and Crystal RG (1998) Regional 'pro-drug' gene therapy: intravenous administration of an adenoviral vector expressing the E. coli cytosine deaminase gene and systemic administration of 5-fluorocytosine suppresses growth of hepatic metastasis of colon carcinoma. Gene Ther 5: 507
102. Tuder RM, Flook BE, Voelkel NF (1995) Increased gene expression for VEGF and the VEGF receptors KDR/flk and flt in lungs exposed to acute or to chronic hypoxia. Modulation of gene expression by nitric oxide. J Clin Invest 95: 1798
103. Wang M, Bronte V, Chen PW, Gritz L, Panicali D, Rosenberg SA, Restifo NP (1995) Active immunotherapy of cancer with a nonreplicating recombinant fowlpox virus encoding a model tumor-associated antigen. J Immunol 154: 4685
104. Wilson JM (1996) Adenoviruses as gene-delivery vehicles. N Engl J Med 334: 1185
105. Worgall S, Wolff G, Falck-Pedersen E, Crystal RG (1997) Innate immune mechanisms dominate elimination of adenoviral vectors following in vivo administration. Hum Gene Ther 8: 37
106. Xiang ZQ, Yang Y, Wilson JM, Ertl HC (1996) A replication-defective human adenovirus recombinant serves as a highly efficacious vaccine carrier. Virology 219: 220
107. Young JW, Inaba K (1996) Dendritic cells as adjuvants for class I major histocompatibility complex-restricted antitumor immunity. J Exp Med 183: 7