Adenovirus-mediated cancer gene therapy and virotherapy (Review)

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Abstract. Gene therapy and virotherapy are among the approaches currently used to treat malignant tumors. Gene therapy and virotherapy use a specific therapeutic gene that causes death in cancer cells. In early attempts at gene therapy, therapeutic genes were driven by ubiquitous promoters such as the CMV promoter, which induce non-specific toxicity to normal cells and tissues in addition to the cancer cells. Recently, novel cancer- and/or tissue-specific promoter systems have been developed to target cancer cells but not normal cells including stem cells. In this review, we describe cancer and/or tissue-specific gene therapy systems for the treatment of cancer. In particular, we will discuss three systems for gene therapy and virotherapy: i) tissue-specific promoter systems, ii) cancer-specific promoter systems, and iii) oncolytic virotherapy. We will also discuss the major challenges of cancer-targeting vector systems and future directions in this area.

Contents

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1. Introduction

Gene therapy and virotherapy are methods to introduce therapeutic genes into cancer cells for the treatment of cancer. The adenoviral vector has been used as a transfer vehicle to introduce genes into cancer cells since it is more efficient than non-viral gene transfer methods (e.g. cationic polymer-DNA complexes) (1,2). The adenoviral vector is stable *in vivo* and efficient in gene delivery to both dividing and non-dividing cells and rarely causes any significant disease itself (3,4). However, one of the limitations of gene therapy using the adenoviral vector is the non-specific expression of therapeutic genes in normal cells causing toxicity to non-cancerous tissues. Targeted expression of therapeutic genes is essential to prevent this toxicity. Recent understanding of tissue- and cancer-specific gene regulation in organogenesis and cancer fields has enabled the development of new promoter/enhancer systems to express therapeutic genes only in targeted cells and tissues. Cancer-specific therapeutic gene expression reduces undesirable toxicity. Thus, adenoviral vectors carrying a cancer-specific promoter system driving a therapeutic gene that is expressed only in cancer cells could be a means to safeguard against unwanted transduction to normal cells and tissues (5,6).

2. Tissue-specific promoter systems

The early adenoviral vectors targeted, not only cancer cells, but also normal cells and tissues since constitutive promoters such as cytomegalovirus (CMV) and Rous sarcoma virus (RSV) were used to drive therapeutic genes (7). The expression of therapeutic genes in unintended, non-targeted normal tissues potentially causes undesirable toxic side effects. To reduce this toxicity, cancer or tissue-specific promoter systems have been developed by replacing constitutive promoters with promoters/enhancers of cancer/tissue-specific gene markers in the adenovirus vector. In this section, we describe tissue-specific promoter systems that target cancers originating from different tissues including prostate, lung, breast, myeloma, and pancreatic cancers.

To target prostate cancer, several prostate tissue-specific promoters including prostate-specific antigen (PSA), probasin (PB) and prostate-specific membrane antigen (PSMA) have been tested for their specificity (8-10). Shi *et al* (11) reported that a helper-dependent adenoviral vector expressing the luciferase gene driven by the PSA promoter/enhancer effectively transduced and expressed luciferase in PSA-positive prostate cancer LNCaP cells. Wu *et al* (12) generated an adenoviral vector expressing the luciferase gene driven by the chimeric PSA enhancer (Ad-PSE-BC-luc). Systemic administration of Ad-PSE-BC-luc into xenografted SCID mice with LAPC-9 human prostate cancer cells resulted in prostate cancer specific luciferase expression while generalized expression occurred using the cytomegalovirus (CMV) promoter driven vector (Ad-CMV-luc). Probasin is also a prostate-specific antigen. Andriani and colleagues (13) generated an adenoviral vector expressing an apoptotic Bax gene driven by the probasin promoter modified to contain two androgen response elements (Av-ARR2PB-Bax). The androgen dihydrotestosterone induced Bax-mediated apoptosis in LNCaP prostate cancer cells after Av-ARR2PB-Bax infection. The vector also exerted an antitumor effect in LNCaP xenograft tumors. Furuhata *et al* (14) generated an adenoviral vector expressing HSV-tk driven by a modified rat probasin (rPB) promoter and found significant growth suppression of the androgen-independent prostate cancer cells in the presence of the prodrug ganciclovir *in vivo*. The modified rPB promoter is able to drive therapeutic genes in response to retinoid in both androgen-dependent and androgen-independent prostate cancer cells and not in other cancer cells or in normal cells.

To target lung cancer cells, the promoters of several types of surfactant protein genes have been tested. Surfactant protein A (SP-A) is expressed in a high proportion of NSCLC tumors with expression limited to the respiratory epithelium (15-18). SP-A can be used as one of the tissue-specific promoters to target lung cancer. Smith *et al* (19) reported that, in plasmid transduction experiments, a luciferase plasmid driven by the 2.75-kb upstream region of the SP-A gene produced significant luciferase activity in SP-A-producing pulmonary H441 adenocarcinoma cells, but only background levels in non-SP-A-producing A549 adenocarcinoma cells. Moreover, the plasmid vector containing HSV-tk driven by the SP-A promoter showed cytotoxic effects in H441 cells but much less in A549 cells. Thus, SP-A promoter elements can be useful for directing the specific expression of a therapeutic gene for the treatment of lung cancer. Surfactant protein B (SP-B) expression is also limited to lung cells (20-23). Strayer *et al* (24) showed that an adenoviral vector that expresses the lacZ gene driven by the 5' flanking region of the SP-B promoter (rAd.SPB.lacZ) targeted H441 and A549 adenocarcinoma cells but not HeLa cervical cancer cells and primary human fetal lung fibroblasts. In contrast, an adenoviral vector that expresses the lacZ gene driven by the constitutive RSV promoter (rAd.RSV.lacZ) targeted all the cells tested.

The MUC1/DF3 promoter has been used to target multiple cancers, including breast cancer, myeloma and pancreatic cancer. MUC1/DF3 is a transmembrane mucin gene normally expressed on the apical borders of secretory epithelial cells. Moehle *et al* (25) found that MUC1 was highly expressed in adult prostate, mammary glands, trachea, lung, small intestine, colon, and in fetal lung. Chen and colleagues (26) generated recombinant adenoviral vectors containing the 5' flanking region of the MUC1/DF3 promoter (-725 to +31), expressing the LacZ gene (Ad.DF3ß-gal) or the herpes simplex virus thymidine kinase (HSV-tk) gene (Ad.Df3-tk). The authors showed that these vectors induced the target gene in MUC1/DF3-positive breast cancer cells but not in MUC1/DF3-negative breast carcinoma cells. Moreover, intraperitoneal injection of Ad.DF3-tk followed by ganciclovir (GCV) treatment resulted in inhibition of tumor growth in an intraperitoneal breast cancer metastases model. Teoh and colleagues (27) analyzed the expression of DF3/MUC1 and the adenovirus receptor CAR in multiple myeloma cells by flow cytometry. They confirmed that the majority of myeloma cells expressed DF3/MUC1 and CAR while normal bone marrow did not express CAR, indicating that an adenovirus vector using the promoter of the DF3/MUC1 gene targets only myeloma and not normal bone marrow. Transduction with the tk gene driven by Ad.DF3-tk followed by treatment with GCV purged multiple myeloma OCI-My5 and RPMI 8226 cells within bone marrow mononuclear cells. Chen *et al* (28) also generated an adenoviral vector expressing the human somatostatin receptor subtype 2 (hSSTR2) gene driven by the 786-bp upstream region of the DF3/MUC1 gene (AdMUC1-hSSTR2). They demonstrated that there was significant inhibition of cell proliferation in DF3/MUC1-positive pancreatic cancer Panc-1 cells. These results demonstrate that utilization of the MUC1/DF3 promoter in an adenoviral vector confers selective expression of target genes in breast cancer, multiple myeloma and pancreatic cancer.

Tissue-specific promoter systems are useful for targeting differentiated cancer. Such systems are not supposed to target normal proliferating tissue, including stem cells. However, there is always a risk of targeting normal differentiated tissue. In the next section, we will discuss another system that targets proliferating cells but not normal differentiated cells.

3. Cancer-specific promoter systems

Five cancer-specific promoter systems utilize genes that are highly expressed in cancer: carcinoembryonic antigen (CEA), α-fetoprotein (AFP), midkine (MK), survivin, and telomerase.

Carcinoembryonic antigen (CEA) is known as an oncofetal protein and adhesion molecule in tumorigenesis. CEA is not usually expressed in normal tissue but it is present in various tumors including gastrointestinal, lung or breast cancer (29,30). The CEA promoter is considered a candidate for gene therapy targeting CEA-positive cancer cells. The cis-acting elements of the CEA promoter for cell-type specific expression were characterized by Schrewe and colleagues (31). Tanaka *et al* (32) constructed a recombinant adenoviral vector expressing HSV-tk driven by the CEA promoter (AdCEA/tk). The cell growth of CEA-producing MKN28 and MKN45 gastric cancer cells infected with AdCEA/tk in the presence of GCV were significantly suppressed while

non-CEA-producing MKN1 gastric cancer cells and HeLa cervical cancer cells infected with AdCEA/tk remained resistant to GCV. Goto *et al* (33) also developed two recombinant adenoviral vectors: one containing the Cre gene under the control of the CEA promoter (AdCEA-Cre) and the other containing the HSV-tk gene (Ad.lox-TK). Coinfection of AdCEA-Cre and Ad.lox-TK followed by GCV administration significantly suppressed the CEA-producing tumor cell-derived peritonitis carcinomatosa in a mouse model. Adenoviral vectors driven by the CEA promoter have been used to target other CEA producing cancers including lung cancer and breast cancer (34,35).

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. For the majority of patients, the current treatments remain unsatisfactory and the prognosis is poor. α-fetoprotein (AFP) is another oncofetal protein. An elevated level of serum AFP is observed in patients with HCC at an advanced stage. Thus, it is one of the diagnostic and prognostic markers of HCC (36,37). Several *cis*- and *trans*-acting elements regulating the human AFP gene have been characterized. Hepatocyte-specific enhancers exist in a distant upstream region (-4.0 kb and -3.3 kb) of the AFP gene (38,39). A hepatocyte-specific silencer was located between the enhancer region and the hepatocyte-specific promoter region (40). Kanai and colleagues (41) developed a recombinant adenovirus expressing the HSV-tk gene containing the human AFP enhancer domain (-4.0 kb and -3.3 kb) and 170 bp of the AFP promoter (Ad.AFPtk) to target AFP-producing HCC cells. The Ad.AFPtk construct along with GCV reduced the cell growth of AFP-producing HuH7 and HepG2 HCC cells but did not affect non-AFP producing HLF HCC cells. Treatment with a recombinant adenovirus expressing the *Escherichia coli* cytosine deaminase (CD) gene driven by the human AFP promoter/enhancer (AdAFPCD) with 5FC also regressed AFP-producing HuH7 and HepG2 HCC cell xenografts (42). Miao *et al* (43) constructed a recombinant adenovirus-Ad/AFPtBid that contained a tBid gene (truncated BH3-interacting domain death agonist) driven by an AFP promoter. Ad/AFPtBid induced apoptosis both in p53-sensitive HCC PLC/PRF/5 cells and p53-resistant HCC Hep3B cells. In *in vivo* experiments, intratumor injection of Ad/AFPtBid significantly inhibited tumor growth of Hep3B cell xenograft tumors in nude mice. These results show the efficacy of the AFP promoter in cancer gene therapy against AFP-producing hepatocellar carcinoma.

Midkine (MK) is a heparin-binding growth factor identified as a product of a retinoic acid-responsive gene. MK has mitogenic activity, anti-apoptotic activity, and angiogenic activity. MK is also involved in oncogenic transformation (44-49). Many human malignant tumors express high levels of MK protein while normal human tissues including liver do not (50). Adachi *et al* (51,52) developed a recombinant adenoviral vector containing the herpes simplex thymidine kinase gene under the control of the 2.3-kb upstream promoter of the human MK gene (AdMKTK). AdMKTK induced marked cell death in response to GCV in G-401 Wilms' tumor cells and SK-N-SH neuroblastoma cells, but did not induce liver toxicity. These findings suggest that the MK promoter may be an important tumor-specific promoter by virtue of its very low hepatic toxicity and high tumor activity.

Survivin is a member of the IAP gene family containing a single baculovirus IAP repeat and no RING finger (53). The expression of survivin is detected in various types of tumors, including non-Hodgkin's lymphoma, breast cancer, lung cancer, ovarian cancer, hepatocellular carcinoma, esophageal cancer and colorectal cancer (53-59) but not in normal tissues including the liver (53,60,61). The survivin promoter is highly active in breast cancer, lung cancer, ovarian cancer and colorectal cancer but inactive in human liver tissue (62-64). These findings suggest that the survivin promoter may be a good candidate to drive therapeutic gene expression for cancer gene therapy. Lu and colleagues (65) constructed recombinant adenoviral vectors expressing the luciferase gene under the control of four different promoters including that of survivin, Cox-2, CXCR4 or EGP-2. Luciferase activity was analyzed in multiple tumor cells including primary melanoma cells and human epithelial melanocytes (HEMs) after infection with these vectors. Among the four promoters, the survivin promoter generated the highest activities in melanoma cells and primary melanoma cells but not in HEMs. Moreover, the survivin promoter showed less activity *in vivo* in major mouse organs including the liver. These data suggest that the survivin promoter may be useful as a tumor-specific promoter with applications for transcriptional targeting of adenovirus vector-based cancer gene therapy.

Telomerase is an RNA-dependent DNA polymerase that synthesizes telomeric DNA. It consists of an RNA component (hTR), a catalytic protein subunit (hTERT), and a telomerase-associated protein TEP1 (66,67). Expression of hTERT is observed at high levels in malignant tumors. There is a strong correlation between hTERT expression and telomerase activity in various types of tumors (68). Takakura *et al* (69) identified an E-box (CACGTG) binding site on the hTERT promoter that is required for c-Myc-mediated hTERT promoter activation. Majumdar *et al* (70) constructed a recombinant adenoviral vector carrying the HSV-tk gene under the control of a 208-bp region upstream of the transcription initiation site of the hTERT promoter (AdhTERT/tk). They also constructed an adenovirus carrying the HSV-tk gene under the control of the constitutive CMV promoter (AdhTERT/tk) to compare with the efficacy of AdhTERT/tk. In a xenograft model using the human 143B osteosarcoma cells, a single injection of both AdhTERT/tk and AdCMV/tk viruses resulted in equivalent tumor regression upon GCV treatment. However, expression of the HSV-tk gene was detected in the liver as well as in tumors in mice injected intratumorally with the AdCMV/tk adenovirus. Expression of the HSV-tk gene by AdCMV/tk in combination with GCV resulted in severe liver histopathology. In contrast, expression of the HSV-tk gene was detected only in tumors and not in the liver in mice with the AdhTERT/tk. These results indicate that the adenovirus system using the hTERT promoter targets only tumors and not normal tissues including liver. Bilsland *et al* (71) constructed adenoviral vectors harboring the suicide gene bacterial nitroreductase (NTR), which bioactivates the prodrug CB1954 into an active cytotoxic alkylating agent under the control of an 876-bp fragment of the hTR proximal promoter (Ad-hTR/NTR) or a 541-bp fragment of the hTERT promoter (Ad-hTERT/NTR). Western blot analysis indicated that NTR expression was

detectable only in cancer cells but not in normal cells after Ad-hTR/NTR or Ad-hTERT/NTR infection. Seven of nine types of cancer cells resulted in up to 18-fold sensitization to the prodrug CB1954 after Ad-hTR/NTR or Ad-hTERT/NTR infection while no sensitization was observed in four types of normal cells. Moreover, an antitumor effect was observed in cervical and ovarian xenograft models following a single intratumoral injection of these vectors, followed by injection with CB1954. These results indicate that the hTERT promoter is a tumor-specific promoter that may be useful for transcriptional targeting for cancer gene therapy.

It has been reported that stem cells, the lymphohematopoietic system, germ cells, and cirrhotic liver have significantly high telomerase activity (72-74). Therefore, the hTERT promoter-mediated gene expression system may cause unknown side effects in these cells and tissues. To develop a promoter system that targets only cancer cells, but not normal cells including stems cells, we recently designed a novel dual tissue- and cancer-specific promoter system by combining the hTERT promoter-mediated cancer-targeted system with a lung-specific tissue-targeted system [TTS system: thyroid transcription factor 1 (TTF1) gene under the control of the hTERT promoter and human surfactant protein A1 (hSPA1) promoter system] (75). We showed that the TTS construct is a lung cancer-specific adenoviral vector system expressing Bax that specifically induces cell death in lung cancer cells including gefitinib (epidermal growth factor receptor tyrosine kinase inhibitor)-resistant cells (6). Because normal cells also proliferate, it is not appropriate for cancer gene therapy to use a promoter such as hTERT that solely targets proliferating cells. Ideally, a promoter system should target cancer cells but not any type of normal cells. Identification and use of appropriate promoter systems that target specific cancer cell types and not normal cells is required for safe cancer gene therapy and virotherapy.

4. Transcriptionally regulated oncolytic adenoviruses

In early gene therapy attempts for the treatment of cancer, viral vectors were used that were modified to delete the function of self-replication in order to achieve safe gene transfer without inducing viral lysis to normal cells and tissues. However, the clinical trials of some suicide gene or corrective gene therapies revealed that the therapeutic effect of non-replicative viruses was limited (76,77). In order to resolve this limitation, conditional replicative adenoviruses (CRAds) were developed for cancer gene therapy, which is also called virotherapy. The conditional replicative viruses are able to replicate and cause cell lysis only in the targeted tumor cells. In addition, the replicated viruses are able to infect neighboring cancer cells and continue this infection cycle until all of the tumor cells are eradicated (78). Virotherapy is the strategy of using a replication-competent virus for cancer therapy. For the safe use of virotherapy, the viruses must replicate only in targeted tumor cells but not in normal cells and tissues.

The ability of adenovirus to transform cells is dependent on the virus-encoded proteins, E1A and E1B, which induce cell cycle progression through the S-phase and provide protection from apoptosis so that efficient virus replication can occur. The deletion of E1A causes the adenovirus to become susceptible to the antiviral mechanisms of the retinoblastoma (Rb) protein by blocking the G1 to S transition. On the other hand, deletion of E1B allows p53 to induce apoptosis in infected cells, aborting replication and spread of the virus because the E1B 55K protein binds p53, targeting it for degradation (79). ONYX-015 is an E1B 55K-deficient adenovirus that contains another viral E1A protein. The ONYX-015 virus is the first conditionally replicating adenovirus that has been tested in a variety of tumor types. Initial preclinical studies demonstrated that ONYX-015 effectively decreased tumor size (80,81). Phase II clinical trials in head and neck cancer patients where intratumoral injection of ONYX-015 in combination with cisplatin and 5-fluorouracil showed a 63% overall response rate and with 27% of patients demonstrating a full clinical response (82).

Another approach for tumor selective virotherapy is the development of an adenoviral vector expressing E1A or/and E1B genes driven by tumor- or tissue-specific promoters and enhancers. This has been achieved in prostate cancer therapies by using the prostate-specific antigen (PSA) promoter. Rodriguez *et al* (83) generated an adenovirus expressing E1A under the control of the 5' flanking region of the human PSA gene (consisting of the enhancer at base pairs -5322 to -3738 fused to the PSA promoter at base pairs -541 to +12) and termed CN706. Immunoblot analysis showed that E1A expression was high in human PSA-producing LNCaP cells but not in PSA non-producing DU145 cells after CN706 infection. The titer of CN706 was significantly higher in LNCaP cells compared to other human PSA non-producing cells (HBL100, PANC1, MCF7, DU145, and OVCAR3). CN706 destroyed LNCaP cell-derived tumors and abolished PSA production in nu/nu mouse xenograft models after a single intratumoral injection. For the treatment of patients with locally recurrent prostate cancer after radiation therapy, a phase I dose-ranging study using an intra-prostatic injection of CV706 resulted in virus dose-dependent reductions in serum PSA levels in treated patients (84).

As we described above, the survivin promoter is a candidate for transcriptional targeting of cancer gene therapy. CRAds containing the survivin promoter have been used to treat glioma and mesothelioma. For the treatment of gliomas, Van Houdt *et al* (85) generated conditionally replicative adenoviruses expressing E1A under the control of the 5' flanking region of the human survivin promoter (a short segment -230 to -130 or a longer segment -1430 to 130) and termed them CRAd-S-S and CRAd-S-L, respectively. These adenoviruses efficiently replicated and killed a variety of established glioma tumor cells but were inactive in a normal human liver organ culture. Furthermore, these CRAds significantly inhibited the growth of glioma xenografts *in vivo* (85). Mesothelioma is a highly malignant neoplasm with no effective treatment. Zhu *et al* (60) constructed a conditionally replicative adenovirus regulated by the survivin promoter with a capsid modification (RGD or F5/3) in the adenovirus fiber region. These CRAd agents effectively targeted human mesothelioma cells and induced strong cytotoxicity in these cells *in vitro* and viral replication in a H226 murine xenograft model *in vivo*. These results suggest that the survivin-based CRAds are promising agents for targeting mesothelioma with

low host toxicity. Together these data indicate that the survivin promoter is a promising tumor-specific promoter for transcriptional targeting of CRAds for cancer.

The cancer-specific midkine promoter has also been used to construct CRAds. Toyoda and colleagues (86) generated the CRAds driven by the 0.6-kb midkine promoter (Ad5MK). Ad5MK showed more cytotoxicity for midkine-expressing Suit-2 and PANC-1 pancreatic cancer cells than for midkine-negative MIAPaCa-2 pancreatic cancer cells. In the Suit-2 cell-derived intraperitoneal xenograft mouse model, the Ad5MK-treated group survived significantly longer than control groups. These studies suggest that a midkine promoter-based conditionally replicative adenovirus might be a promising new virotherapy for pancreatic cancer.

In our own research we also used CRAd to target mesothelioma (6,75,87). Gordon and colleagues (88) profiled the gene expression pattern of malignant pleural mesothelioma, normal lung, and pleural tissues using cDNA microarrays. They reported that the CRI1 (CREBBP/EP300 inhibitory protein 1) gene was a specific marker for malignant pleural mesothelioma. We made a construct containing four tandem repeats of the CRI1 promoter-138/-1 (CRI1-138 4x) and observed that the use of the tandem repeats caused significantly higher promoter activity in malignant pleural mesothelioma cells but little promoter activity in normal mesothelial cells and normal fibroblasts. We developed a CRAd-expressing E1A driven by the CRI1-138 4x promoter system (Ad-CRI$^{-138\ 4x}$/E1A). Ad-CRI$^{-138\ 4x}$/E1A induced viral proliferation and cell death only in a mesothelioma specific manner and showed antitumor effects in a mesothelioma xenograft mouse model (87). These results suggest that CRI1-138 4x is a promising promoter system to use in CRAd to target mesothelioma.

In earlier cancer gene therapies using adenovirus, anti-apoptotic genes, such as Bax, tBid and HSV-tk were used to cause cell death in cancer cells. However, cell death occurred only in virus-injected cancer cells but not in the non-injected neighboring cancer cells. In order to solve this limitation, CRAds were developed, which spread to all cancer cells even non-injected ones. The development of cancer type-specific promoters is still required to avoid targeting normal cells, but viral proteins including E1A causing cell lysis/death to all cancer cells will be employed for future cancer gene therapy.

5. Major challenges of cancer-targeting vectors

One of the major challenges in adenovirus-mediated cancer gene therapy and virotherapy is poor transduction in human tumors. The reason for this poor transduction is that tumor cells have limited surface expression of the Ad5 primary receptor, the coxsackievirus and adenovirus receptor (CAR) that are necessary for transduction. To resolve the problem, several modifications to the adenovirus fiber have been performed. Wickham *et al* (89) constructed adenoviral vectors, which contain modifications to the adenoviral fiber coat protein that redirect virus binding to either α(v) integrin [AdZ.F(RGD)] or heparan sulfate [AdZ.F(pK7)] cellular receptors. They reported that AdZ.F(RGD) increased gene delivery to endothelial and smooth muscle cells expressing α(v) integrins and that AdZ.F(pK7) increased transduction 5- to 500-fold in multiple cell types lacking high levels of the adenoviral fiber receptor. Another approach is to create chimeric viruses, usually based on adenovirus serotype 5 (Ad5). The fiber or knob domain is replaced by that of another serotype Ad5 with adenovirus serotype 3 (Ad3). This virus showed CAR-independent infectivity (90,91). Ulasov and colleagues (92) have shown that a chimeric Ad5/3 vector which contains the shaft of adenovirus serotype 5 and the knob of adenovirus serotype 3 can target the CD46 cellular receptor and increase transduction of glioma cells. Kanerva *et al* (93) reported that the Ad5/3 vector increased transduction of ovarian cancer cells. Similarly, adenoviral vectors constructed with the serotype 17 fiber (Ad17) improve transduction of airway epithelial tissue. Adenoviral vectors with serotype 35 (Ad35) improve transduction of hematopoietic cells (94,95). Krasnykh *et al* (96) replaced the fiber and knob domains with bacteriophage T4 fibritin proteins. This modified adenovirus lacks the ability to interact with CAR and demonstrates much higher reporter gene expression in HEK293 cells expressing a 6-His-binding receptor.

Immune responses against the adenovirus drastically limit the vector transduction efficiency and the duration of transgene expression. In order to overcome this issue several strategies have been examined. Immunosuppressive agents including cyclosporin, FK506 or anti-CD4 monoclonal antibody have been found to enhance transgene expression of adenoviral vectors (97-99). Chirmule and colleagues (100) treated rhesus monkeys with CD40 ligand antibody (hu5C8) after inoculating the lung with adenoviral vector. Immunological analyses demonstrated suppression of adenovirus-induced lymphoproliferation and cytokine responses (IL-2, INF-γ, IL-4, and IL-10) in the hu5C8-treated rhesus monkeys. The hu5C8 treatment resulted in significant and prolonged inhibition of the adenovirus-specific humoral response well beyond the time when hu5C8 effects were no longer significant.

Alteration of the immunodominant epitopes of the adenoviral capsid is also helpful in evading immune responses. Covalent attachment of polymers to adenoviral capsid protein has been shown to curtail antibody-mediated virus neutralization. O'Riordan *et al* (101) showed that PEG-modified adenovirus can be protected from antibody neutralization in the lungs of mice with high antibody titers to adenovirus, suggesting that PEGylation improves the ability to administer adenoviral vectors. Fisher *et al* (102,103) reported that incorporation of targeting ligands such as basic fibroblast growth factor and vascular endothelial growth factor on N-(2-hydroxypropyl) methacrylamide (HPMA)-coated virus produces ligand-mediated CAR-independent binding and uptake into cells which have appropriate receptors. Serotype switching in vector construction is another method to evade the vector immune response in human gene therapy since the neutralizing immune response to adenovirus is serotype-specific. The replication-defective Ad11 or Ad35 vectors have been reported to elude preexisting adenoviral immunity (104,105). In addition, Parks *et al* (106) showed that helper dependent adenoviral vectors elicited a limited cell-mediated immune response without causing significant liver damage and toxicity. Roberts and colleagues (107) have reported the construction of a novel hexon-chimeric adenoviral vector that circumvents preexisting anti-vector immunity. These new

vectors combined with CRAds whose expression is driven by cancer cell-type specific promoters will enable the targeting and destruction of cancer cells without affecting normal cells including stem cells.

6. Conclusions

Adenoviral vectors are the most promising and widely used platform for gene therapy and virotherapy. However, there have been problems associated with their use. Recently researchers have been working to improve the efficacy of these vectors by focusing on improving three parts of the vector systems. One goal is to obtain specificity to cancer cells in order to avoid damaging normal cells. The promoter design to acquire cancer cell specificity has evolved from the earlier constitutive promoter system to the cancer/tissue targeted promoter systems. A second goal is to improve the means to induce cancer cell death. The modification of viral proteins to cause cell death/lysis in cancer cells while also promoting viral replication in other cancer cells but not in normal cells has been realized. This breakthrough will enable the targeting not only of neighboring cancer cells, but also metastasized cancer cells. A third goal is to improve transduction. The surface structure of adenovirus vectors has been modified to deliver therapeutic genes into cancer cells more efficiently. Furthermore, some of the modifications enable the virus to avoid the immune response of the patient. These cancer-specific adenovirus vectors equipped with an efficient delivery system are ready to be used immediately in *in vivo* mouse models and tested for clinical trials.

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