Targeting of cancer gene therapy with antibodies or their genes against tumor-associated antigens

Review Article

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Abbreviations: adenoviral vectors, (adenovectors); carcinoembryonic antigen, (CEA); coxsackie-adenovirus receptor, (CAR); epidermal growth factor receptor, (EGFR); epithelial cell adhesion molecule, (EpCAM); high-molecular weight melanoma-associated antigen, (HMWMMAA); inducible nitric oxide synthase, (iNOS); matrix metalloprotease, (MMP); murine leukemia virus, (MLV); nitric oxide, (NO); retroviral vectors, (retrovectors); arginine-glycine-aspartic acid, (RGD), single-chain diabody, (scDb); single-chain variable fragmented antibodies, (scFvs); severe combined immunodeficiency, (SCID); surface domain, (SU); tumor-associated antigens, (TAAs)

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Summary

Gene therapy is expected to play a major role in future cancer treatment. Actually various therapeutic genes have shown promise for tumor cell killing. However, successful gene therapy depends on the development of efficient and targeted gene transfer vectors. This overview summarizes the current use of anti-tumor-associated antigen (TAA) antibodies in cancer gene therapy. Current data suggest that antibodies or their genes against TAAs can be used for targeting viral vectors for cancer gene therapy.

I. Introduction

Tumor-associated antigens (TAAs) are molecules which occur in or on tumor cells and are either not demonstrable or are significantly less abundant in normal tissues (Groen, 1987). There are many TAAs against which monoclonal antibodies are now utilized for immunotherapy of cancer (Kuroki et al, 2002).

The strategies of gene therapy for cancer can be largely categorized into either direct or indirect gene therapy (Kuroki et al, 2002). Direct gene therapy for cancer involves the insertion of a gene to tumor cells for the direct killing or suppression of abnormal growth by gene products or their secondary products. The genes used for this strategy are suicide genes, functioning tumor suppressor genes, anti-sense genes against known oncogenes, or antiangiogenic genes (Kuroki et al, 2000). Indirect gene therapy involves the insertion of a gene that modifies or stimulates immunocytes to be more effective against tumor cells. Recent knowledge that T cell-recognized peptide epitopes are presented by HLA molecules, and that the induction of immune responses is dependent on co-stimuli, has led to the development of more rational strategies (Roitt et al, 1998). The genes used for this strategy are cytokine genes, TAA genes, costimulatory molecule genes, or HLA class I genes, which are often inserted into tumor cells as well as immunocytes (Kuroki et al, 2000).

Viral vectors have been extensively used for cancer gene therapy because of their relatively high efficacy of gene transfer (El-Aneed, 2004). Retroviral vectors (retrovectors) and adenoviral vectors (adenovectors) are among the most frequently chosen vector systems (Hunt et al, 2002). However, these vectors still have several specific problems regarding their pathogenicity (immunogenicity), their gene transfer efficacy, the stability and level of transgene expression, a limitation in terms of the size of the inserted gene, and a limitation in specifically targeting tumor cells, etc (El-Aneed, 2004; Hunt et al, 2002). Among them, the biggest problem is probably the lack of tumor specificity of viral vectors used.
for gene transfer (Kuroki et al, 2000; Dachs et al, 1997). In this regard, genetically engineered single-chain variable fragmented antibodies (scFvs) or their genes to TAAs have recently been used for increasing tumor specificity of viral vectors (Kuroki et al, 2003). This article provides a brief overview of the tumor targeting strategies of retrovectors and adenovectors for cancer gene therapy by using antibodies or their genes against TAAs.

II. Tumor targeting of retrovectors

Retrovectors remain an attractive option for clinical gene delivery because integration of the vector genome allows stable gene expression in the infected cell and its progeny (Hunt et al, 2002). The retrovectors used for most clinical trials of gene therapy originate from a murine leukemia virus (MLV). Because viral coding regions are deleted from the vector, viral proteins are not expressed in the infected cells, avoiding stimulation of an inappropriate immune response. Also, the host range of retrovectors is usually determined by the surface domain of the envelope glycoprotein, which covers the viral capsid and binds to a cell surface receptor. As retrovectors transduce only dividing cells, they have been used to deliver therapeutic genes to tumors in vivo, with surrounding normal tissue being largely refractory to transduction (Chowdhury et al, 2004).

Recently, however, a patient with X-linked severe combined immunodeficiency (SCID), who received gene therapy using retrovirally transduced bone marrow cells, developed T cell leukemia caused by retrovector integration leading to insertional mutagenesis (Kohn et al, 2003). This highlights the need to target retroviral gene delivery specifically to tumors, if vectors or packaging cells are to be injected in vivo for cancer gene therapy. Most tumors induced by retrovectors involve hematopoietic cells transformed by insertional mutagenesis. Thus, particular care is needed to avoid transduction and potential transformation of these cells. To establish gene therapy as a feasible treatment of cancer, more emphasis is required on developing optimal gene delivery systems with a greater tumor tissue specificity. One of the efforts of tissue-specific targeting is based on attempts to engineer the normal retroviral envelope protein (Kuroki et al, 2003). Recent advances in the field of genetic engineering have led to development of a concept for target cell specificity by modifying the tropism of the normal envelope, retroviral receptor-binding domain with an scFv antibody or a ligand that recognizes a TAA (Russell et al, 1993; Somia et al, 1995) or a specific cell surface receptor (Kasahara et al, 1994). We focus here on the strategy using anti-TAA scFv antibody genes for increasing the tumor specificity of retrovectors. The major antibody-recognized TAAs currently used as the targets are carcinoembryonic antigen (CEA) (Chowdhury et al, 2004; Konishi et al, 1998; Khare et al, 2001 and 2002) and high-molecular weight melanoma-associated antigen (HMWMAA) (Martin et al, 2002 and 2003).

In a recent study, we developed a novel bifunctional MLV-based recombinant retrovector that displays a chimeric envelope protein containing an scFv antibody to CEA and carries a suicide, inducible nitric oxide synthase (iNOS) gene in the genome (Figure 1) (Khare et al, 2001).

![Figure 1](image-url). Specific targeting of retrovector carrying a suicide gene (the iNOS gene) to CEA-expressing tumor cells with a chimeric envelope protein containing an anti-CEA scFv antibody.
The MLV-based retrovector used here is ecotropic, and originally infects only murine cells. CEA is expressed by a number of tumors of epithelial origin, most notably colorectal carcinoma. The iNOS gene product yields nitric oxide (NO), which directly induces autolysis and cytolytic of by-stander cells. An anti-CEA scFv antibody gene derived from the mouse hybridoma F11-39 was genetically inserted between the sixth and seventh amino acid of the ectropic envelope. The resultant bifunctional retrovector, GPEscFv-env/iNOS, showed a specific delivery of the iNOS gene to human CEA-expressing tumor cells (MKN-45 gastric carcinoma cells) and directly and efficiently killed the infected CEA-expressing tumor cells by the induction of apoptosis without any additional drugs. The targeted vector was able to produce tumor suppression in a SCID mouse xenograft model with a 70% reduction in tumor weight (Khare et al, 2002).

In a previous study, Martin et al, (1999) described retrovectors targeted to HMWMMA, which is expressed in more than 90% of human melanomas. The chimeric envelope surface domain (SU) contained an scFv recognizing HMWMMA followed by a proline linker and a matrix metalloprotease (MMP) cleavage site. The proline linker prevented binding of the chimeric SU to its Pit-2 receptor. However, when these vectors bound to HMWMMA, they were then cleaved by cell surface MMPs, revealing the amphotropic 4070A (MLV-A) backbone that mediated transduction via the Pit-2 receptor. The targeted vector (LMH2/ProMMP) infected HMWMMA-positive cells when injected into a nude mouse xenograft model (Martin et al, 2002). Recently, they also reported a new retrovector targeted to CEA using the MFE23 scFv antibody against CEA (Chowdhury et al, 2004). The envelope MFE23/ProMMP was constructed by linking MFE23 to the amino terminus of MLV-A SU using a proline-rich spacer followed by a cleavage site for MMPs. Retrovectors incorporated the MFE23/ProMMP envelope as efficiently as the unmodified MLV-A envelope, in contrast to the relatively poor incorporation of many chimeric envelopes (Martin et al, 1999), and could specifically transduce CEA-positive cells or tumors with high efficiency (Chowdhury et al, 2004).

Taken together, these results suggest that a tumor-specific therapeutic effect could be achieved by using the scFv-chimeric retroviral envelope protein model to deliver suicide genes in vivo and this approach could also be applied to other TAAs expressing on cancer cells.

### III. Tumor targeting of adenovectors

Adenovectors are also promising reagents for clinical gene delivery because of their superior in vivo gene transfer efficiency on a wide spectrum of cell types and their low risk of mutagenesis. Adenovectors, like adenoviruses, do not have an envelope and their major capsid components are hexon, penton (or penton base), and knobbed fiber (fiber and fiber knob). Adenoviral infection is mediated by binding of the knob region, located at the carboxy terminus of the fiber, to its corresponding receptor, which is the coxsackie-adenovirus receptor (CAR) (Bauerschmitz et al, 2002). Binding is followed by interaction between cellular integrins and an arginine-glycine-aspartic acid (RGD) motif located at the penton base. Infection is not dependent on cell cycle phase; therefore, both cycling and non-dividing cells are infected, and adenoviral DNA is not integrated into the host genome. Nevertheless, the limited duration of gene expression may render adenovectors less desirable for the gene therapy of hereditary diseases where long-term expression is needed, but it is adequate for cancer gene therapy approaches where the primary purpose is to kill the target cells (Bauerschmitz et al, 2002).

However, adenovectors should also possess critical properties required for the development of efficient and targeted gene transfer vectors for the successful clinical translation of cancer gene therapy (Nettelbeck et al, 2004). These include a highly evolved gene transfer mechanism, the stability of virus particles and the ease of virus production at high titers. The necessity of such improvement is predicated by the observation that CAR is widely expressed on normal tissues resulting in nonspecific susceptibility to adenoviral infection. In addition, reduced or absent expression of CAR has been reported for various tumor types, indicating resistance to adenoviral infection by tumor cells in situ. These considerations of adenoviral biology are paralleled by the observation of limited efficacy and vector-related toxicity in preclinical and clinical adenoviral gene therapy studies. Therefore, the development of tropism-modified, tumor-targeted adenovectors is a key endeavor in current gene therapy approach. To this end, the native tropism of adenoviruses needs to be ablated and a new, tumor-specific tropism needs to be engineered into viral particles (Nettelbeck et al, 2004). The trial has been performed in several ways: a) fusion protein of soluble CAR (sCAR) and targeting-receptor ligand (Dmitriev et al, 2000), b) fusion protein of anti-fiber knob antibody and targeting-receptor ligand (Watkins et al, 1997), c) bispecific antibody to fiber knob and TAA (cell receptor) (Haisma et al, 1999; Nettelbeck et al, 2001), d) fusion protein of sCAR and scFv antibody to TAA (Kashentseva et al, 2002), and e) immunoglobulin-binding domain inserted fiber-knob protein (Volpers et al, 2003), etc. Here we focus on the last three strategies that have been utilizing anti-TAA (or anti-cell receptor antibodies) or their genes for increasing tumor specificity of adenovectors. The antibody-recognized TAAs (or cell receptors) used as the targets are HMWMMA (Nettelbeck et al, 2004), epithelial cell adhesion molecule (EpCAM) (Haisma et al, 1999; Heideman et al, 2001), epidermal growth factor receptor (EGFR) (Volpers et al, 2003; Haisma et al, 2000), HER-2 (Her-2/neu or c-erbB-2) (Kashentseva et al, 2002), CD-40 (Korokhov et al, 2003), CD-70 (Israel et al, 2001), and CD-105 (Nettelbeck et al, 2001), etc.

In previous studies, Haisma et al (1999) and Heideman et al (2001) demonstrated tumor-specific gene transfer via an adenovector targeted to the pan-carcinoma antigen EpCAM. An anti-fiber knob Fab' antibody conjugated to an anti-EpCAM Fab' antibody was created that targets the adenovirus to the EpCAM antigen present on tumor cells. The EpCAM antigen was chosen as the target because this antigen is highly expressed on a variety of adenocarcinomas of different origin such as breast.
In these studies, the EpCAM-targeted adenovector was shown to specifically infect cancer cell lines of different origin expressing EpCAM. Gene transfer was blocked by excess anti-EpCAM antibody and dramatically reduced in EpCAM negative cell lines, thus showing the specificity of the EpCAM-targeted adenovector. Importantly, infection with targeted adenovector was independent of CAR, which is the natural receptor for adenovirus binding, since blocking of CAR with recombinant fiber knob did not affect infection with targeted adenovirus. Apart from the cancer cell lines, the efficacy of targeted viral infection was studied in freshly isolated primary human colon cancer cells. As colon cancer predominantly metastasizes to liver, and adenovirus has a high tropism for hepatocytes, they determined if the EpCAM-targeted adenovector showed reduced infectivity of human liver cells. The bispecific antibody could successfully mediate gene transfer to primary human colon cancer cells, whereas it almost completely abolished infection of liver cells. Thus, chemically prepared bispecific antibodies are versatile tools, but the production and purification of the conjugates poses problems of heterogeneity and is time consuming. More recently, Nettelbeck et al, (2004) reported retargeting of adenoviral infection to melanoma by combining genetic ablation of native tropism with a recombinant bispecific single-chain diabody (scDb) adapter that binds to fiber knob and HMWMAA. This strategy combines genetic ablation of native adenoviral tropism with redirected viral binding to melanoma cells via a bispecific adapter molecule, a bacterially expressed single-chain diabody, scDb MelAd, that binds to both the adenoviral fiber knob and to HMWMAA. The results showed specific and strong binding of the bispecific adapter scDb MelAd to melanoma cells. In adenoviral infection experiments, they demonstrated a) substantially reduced infectivity of capsid mutant adenoviruses, b) restored, CAR-independent and HMWMAA-mediated infectivity of these mutant viruses by scDb MelAd specifically in melanoma cells, and c) higher levels of transgene expression in melanoma cells by fiber mutant virus complexed with scDb MelAd, relative to a vector with wild-type fibers. Hence, the HMWMAA-targeted adenovector lacking native tropism exhibits both enhanced specificity and augmented infectivity of gene transfer to melanoma cells, suggesting that it is feasible to use this vector to improve gene therapy for malignant melanoma.

On the other hand, Kashentseva et al, (2002) have proposed the use of the sCAR ectodomain fused with a ligand to block CAR-dependent native tropism and to simultaneously achieve infection through a novel receptor overexpressed in target tumor cells. To confer adenovector-targeting capability on cancer cells expressing the HER-2 oncogene, they engineered a bispecific adapter protein, sCARIC6.5, that consisted of sCAR, phage T4 fibrin polypeptide, and the C6.5 scFv antibody against HER-2 oncoprotein. They demonstrated that the sCARIC6.5 protein binds to cellular HER-2 oncoprotein and mediates efficient adenovector targeting via a CAR-independent pathway. Targeted adenovector, complexed with sCARIC6.5 adapter protein, provided significant enhancement of gene transfer compared with adenovector alone and untargeted adenovector complexed with CAR control protein. Thus, the use of recombinant trimeric sCAR-scFv adapter proteins may augment adenovector potency for targeting cancer cell types.

In addition, Volpers et al, (2003) developed a novel modified adenovector that displays a synthetic IgG-binding domain in the capsid and carries a reporter lacZ gene (Figure 2).

Figure 2. Specific targeting of adenovector carrying a reporter gene (the lacZ gene) to EGFR-expressing tumor cells with a chimeric fiber-knob protein containing an immunoglobulin-binding domain (Z33).
A synthetic 33-amino-acid IgG-binding domain (Z33), derived from staphylococcal protein A, was inserted into the adenovirus fiber protein. The fiber retained the ability to assemble into trimers, bound IgG with high affinity, and was incorporated into vector particles. The transduction efficiency of the Z33-modified adenovector in human EGFR-expressing tumor cells (A431 epidermoid carcinoma cells) was strongly and dose-dependently enhanced by combination with an EGFR-specific monoclonal antibody. The antibody-mediated increase in cellular transduction was abolished in the presence of competing protein A. More recently, Henning et al. (2005) constructed two kinds of adenovirus 5 vectors carrying knobless fibers with antibody-binding domains from Staphylococcal protein A or from Streptococcal protein G, respectively. Both adenovectors bound their specific Ig isotypes with the expected affinity. They transduced human carcinoma cells independently of the CAR pathway, via cell surface receptors targeted by specific monoclonal antibodies, that is, EGFR expressed on A549, HT29 and SW1116, HER-2/neu on SK-OV-3 and SK-BR-3, CA242 antigen on HT29 and SW1116, and prostate-specific membrane antigen on HEK-293 cells, respectively. Thus, the antibody-binding adenovector also holds promise for directed gene transfer to a wide variety of cell types by simply changing the target-specific antibody.

IV. Conclusion
Cancer gene therapy is one of the main applications of gene therapy. In the past decade, both viral and nonviral vectors have been developed and evaluated for delivering therapeutic genes that can eliminate tumor cells. In the last few years, numerous modifications to the delivery systems have been made to optimize the transfection efficacy. Among them, the strategies to target viral vectors to tumor tissues by modifying the tropisms with antibodies or their genes against TAAs are very promising from a practical point of view.

References

First row from left to right: Jian Huang, Ken Hachimine, Hirotomo Shibaguchi, Masahide Kuroki
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