Gene Therapy

AIDS-Related Malignancies

As in other immunodeficiency states, the risk of certain malignancies is higher than average in HIV infection. Up to 25% of all cancers in men under the age of 45 in the United States are associated with HIV infection, and as many as 30% of all HIV-infected persons will ultimately develop cancer. Non-Hodgkin's lymphoma, Kaposi's sarcoma, and cervical carcinoma are AIDS-defining, and the incidence of other malignancies is likely increased as well.

A modest literature exists on gene therapy for AIDS-related malignancies, and the field is advancing. However, the newly formed AIDS Malignancy Consortium is currently sponsoring no trials of gene therapy for any AIDS-related malignancy, nor is the AIDS Clinical Trials Group. Given the modest success of gene therapy in other contexts, though, the current status of gene therapy for AIDS-related malignancy may be reasonable.

A growing concern is that the number of AIDS-related malignancy cases will increase as HIV-infected individuals live longer. Recognizing this, the National Cancer Institute sponsored its first meeting devoted entirely to AIDS-related malignancy in April 1997. Although the meeting attracted a worldwide audience, few talks or abstracts were devoted to gene therapy.

AIDS-related malignancies are usually incurable and often difficult to treat, but many of them are virus-related or require specific growth factors. Gene therapy represents an attractive strategy to augment the therapeutic armamentarium, provided that clinically effective, safe, tolerable, convenient, and cost-effective approaches are developed.

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Kaposi's sarcoma (KS) develops from a cell of uncertain origin and is almost always associated with infection with the recently described human herpes virus 8 (HHV-8 or KSHV). Chemotherapy for systemic disease has enjoyed some success but is not curative and is often toxic.

KS tumors produce an extraordinary variety of growth factors, and their growth may also be hormonally influenced. Some of the growth factors enhance tumor cell but not normal endothelial cell proliferation and thus may serve as targets for specific attack. Antisense oligodeoxynucleotides directed against oncostatin M, basic fibroblast growth factor, or c-*myc* have all treated KS lesions in vitro or in animal models.

The HIV Tat protein has been implicated in the pathogenesis of Kaposi's sarcoma. Gene therapies against Tat have inhibited HIV itself, and may be tested in KS as well. Newer generation adenoviral vectors are being constructed with higher affinity for KS and better gene expression in these tumors.

The precise role of HHV-8 in the pathogenesis of KS remains to be elucidated. However, the viral genome encodes a variety of proteins that are homologues of mammalian cytokines, cytokine receptors, cell cycle regulators, or other proteins involved in cell growth and differentiation, all of which are possible targets for gene therapy-mediated inhibition. The interferon releasing gene product may be a particularly promising target, as it appears to be involved in tumorigenesis in a mouse model.

Anti-angiogenesis gene therapies that prove efficacious in other contexts may be effective in KS treatment as well, because this tumor is highly dependent on angiogenic growth factors.

Progress in anti-KS research is hampered by the lack of suitable cell lines for study. Whereas several non-Hodgkin's lymphoma cell lines infected with HHV-8 are now available, no HHV-8-infected KS cell line has been reported. Cultivation of HHV-8 in vitro has recently been described, though, and will likely be of considerable help in KS research.

Non-Hodgkin's lymphomas are primarily B cell tumors, and there are three distinct types related to AIDS: systemic, primary CNS, and primary effusion lymphomas. Approximately half of the systemic lymphomas are Epstein-Barr virus (EBV)-transformed, as are virtually all of the primary CNS and primary effusion lymphomas. In addition, primary effusion lymphomas are essentially all infected with HHV-8.

The Epstein-Barr virus genes expressed in non-Hodgkin's lymphoma offer specific sites for attack by gene therapies. HHV-8 lytic or latent genes may also be suitable targets in infected cells, although little is known regarding the role of HHV-8 in lymphomagenesis. In addition, most non-Hodgkin's lymphomas, except the primary effusion lymphomas, express a variety of antigens specific to B cells and encode B cell-specific or EBV-specific gene regulatory elements.

Four possible gene therapies capitalizing on these targets have been described: (1) antisense oligodeoxynucleotides against EBV transforming genes, which chemosensitize tumors and prolong survival in mice with severe combined immunodeficiency disease (SCID); (2) intracellular antibodies against

the EBV transforming gene *LMP-1*, which also chemosensitizes tumors; (3) immunoglobulin-regulated toxin genes, which cure some non-Hodgkin's lymphomas in SCID mice when delivered via an adenovirus-polylysine vector; and (4) EBV-regulated toxin genes, which treat non-Hodgkin's lymphoma in SCID mice.

Among other reported strategies is expression of conditional toxin genes, such as herpes simplex thymidine kinase, which makes non-Hodgkin's lymphoma cells sensitive to ganciclovir. Antisense oligodeoxynucleotides (ODN) against human interleukin 10 inhibit the growth of non-Hodgkin's lymphoma cell lines, and antisense ODN against HHV-8 IL-10 inhibit the growth of primary effusion lymphomas in vitro.

Complexes of anti-B cell antibodies and toxins have also been tested. Although this work is not strictly gene therapy, it bears mentioning because these conjugates are effective in animal models and are generally safe and well tolerated in Phase I human clinical trials. Synergy with chemotherapy has also been demonstrated in animals.

Whereas heterologous gene expression in Kaposi's sarcoma cells has proved relatively easy, it has been notoriously difficult to achieve high-level gene expression in EBV-transformed non-Hodgkin's lymphoma. My colleagues and I have demonstrated that adenovirus-polylysine complexes are efficient in this regard and that gene expression may be improved by incorporating an anti-immunoglobulin into the complex. We also recently reported on a newer generation of adenovirus vectors with enhanced ability to effect gene expression in EBV-transformed tumors in vitro.

There are no published reports on gene therapy for cervical carcinoma in the setting of HIV disease. Most cervical cancers are related to infection with human papilloma virus (HPV), affording a site of specific attack. Research in this area has been slowed by the inability to grow HPV in culture.

Antisense oligodeoxynucleotides directed against the HPV oncogene products E6 or E7, and sense ODN encoding the retinoblastoma gene product, have been used in vitro to inhibit growth of E6 or E7-positive cervical carcinoma cells. Antisense ODN against E7 also inhibited HPV-transformed tumor cells in vitro.

Antisense ODN directed against HPV E6 or E7 oncoproteins successfully treated HPV-transformed cervical cancer cells in nude mice. Adeno-associated viral vectors encoding a synthetic interferon gene partially treated tumor-bearing mice. An antisense-encoding plasmid that interfered with production of folate receptors retarded cervical cancer tumors both in vitro and in vivo. Transfer of a wild-type *p53* gene into cervical carcinoma cell lines using an adenoviral vector strongly induced tumor apoptosis.

Cervical tumors are anatomically easily accessible, and epithelial cells are easily transducible by several vector systems. Gene therapy approaches using adenoviral vectors, which transduce genitourinary tract epithelial cells with extremely high efficiency, may be assessed in this disease.

Although HIV is a lytic rather than a transforming virus, it may contribute to oncogenesis by a variety of mechanisms. HIV-infected stromal cells may augment the growth of non-Hodgkin's lymphoma. HIV Tat may play a role in Kaposi's sarcoma. Activated T cells secrete cytokines that may contribute to development of either of these malignancies, and HIV-induced immunodeficiency may increase cancer risk.

In fact, epidemiologic data now suggest that the risk for developing Hodgkin's disease, anal squamous cell carcinoma, oropharyngeal carcinoma, seminoma, myeloma, melanoma, and conjunctival squamous cell carcinoma may be increased 3 to 70 fold in HIV infection. The risk for leiomyosarcoma in children may be even higher. Gene therapy that decreases HIV viral burden may therefore hinder the development or progression of cancers and may be viewed as an indirect approach to AIDS-related malignancy.

Theoretically, any available gene therapy technique may be used in treatment of AIDS-related malignancy. Live, attenuated retroviruses and adenoviruses are among the most efficient vectors for human gene therapy, but because of the immunocompromised state of many HIV-infected patients, their use in such patients may pose substantial safety risks. Nonetheless, attenuated live viral vaccines have been given safely to some HIV-infected patients, and their use for AIDS-related malignancy should not be discounted, although prudence must be exercised.

Strategies to enhance the immunogenicity of AIDS-related tumors in immunodeficient hosts have not been pursued because of their low likelihood of success. On the other hand, the possibility of a counterproductive immune response against gene therapy vectors or products may be lower.

The metabolism or pharmacokinetics of some drugs may be altered in HIV infection. Furthermore, HIV-infected individuals have a propensity for adverse drug reactions. The potential adverse effects on gene therapy for AIDS-related malignancies have not been well defined.

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