Gene Therapy

Radiation Therapy and Gene Therapy

In the treatment of neoplastic diseases, external-beam radiation therapy is limited by toxicity from irradiation of normal tissues and radioresistance of many malignancies to conventional radiation doses. Radioimmunotherapy, in which radiolabeled monoclonal antibodies target tumor-associated antigens or receptors, is limited by poor tumor antigen or receptor expression, leading to low tumor localization of the antibody.

Gene therapy offers approaches to overcome these limitations. Gene transfer strategies can be used to (1) increase the radiosensitivity of cancer cells, (2) increase the expression of antigens or receptors on tumor cell surfaces, or (3) produce toxic and radiosensitizing drugs in cancer cells.

Potential targets for increasing the radiosensitivity of cancer cells by gene transfer are factors that control the response of cells to radiation. These include enzymes that control cell cycle progression, genes that control the induction of apoptosis, DNA repair enzymes, oncogenes correlating with radioresistance, and growth factor receptors involved in cell signaling pathways.

Mammalian cells are most radiation-sensitive in mitosis and somewhat less sensitive in G_2 and at the G_1/S phase boundary. Cyclin B is important for G_2 progression, and cyclins D and A are important for traversal of G_1 and progression into S phase, respectively. Abrogation of the cyclin genes may radiosensitize by arresting tumor cells in sensitive cell cycle phases.

Radiation causes apoptosis in a variety of human tumor cell lines. The *bcl-2/bax* gene family includes genes that prevent apoptosis and others that induce it. An even balance between pro-apoptotic and anti-apoptotic genes maintains cell viability. Overexpression of *bax* or other pro-apoptotic genes or abrogation of *bcl-2* or other anti-apoptotic genes may upset this balance and induce apoptosis, thus increasing cellular radiation sensitivity.

Mutations in DNA repair genes increase the radiation sensitivity of mammalian cells. Abrogation of repair pathways by gene transfer may sensitize human cancer cells, as it does in DNA repair-mutant cell lines. Examples of DNA repair genes and their products are the double-strand binding Ku70/80 proteins, the ataxia-telangiectasia gene, DNA-dependent protein kinase, and human homologues of yeast Rad 50 series repair proteins. Genetic knockout of these DNA repair proteins may create radiosensitive mutants of human tumor cells.

Interference with growth signaling pathways may also lead to increased radiation sensitivity. Potential targets are the epidermal growth factor receptor family, including erbB-2. We used an intracellular single-chain antibody to erbB-2 to down-regulate cell surface expression of erbB-2, induce apoptosis, and thereby radiosensitize human ovarian cancer xenografts in mice.

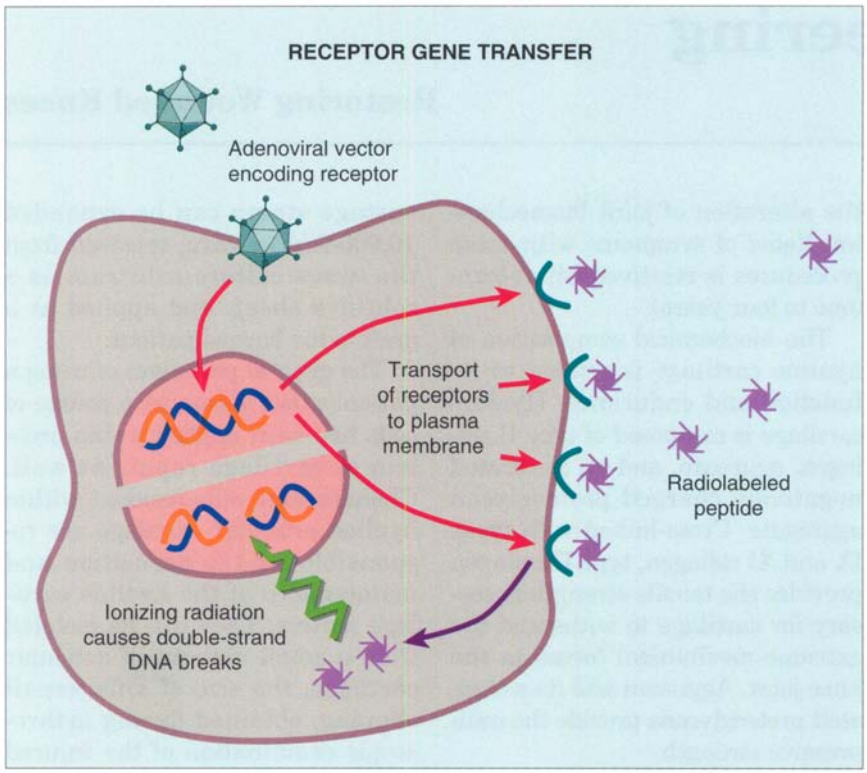
Other investigators have used antisense oligodeoxynucleotides or ribozymes to inhibit the expression of several oncogenes, including *ras*. Overexpression of the *ras* and *myc* oncogenes correlates with increased radiation resistance of cells. Abrogation of oncogenes may radiosensitize tumor cells.

Mutation compensation restores the normal function of a mutated tumor suppressor gene by incorporating a normal gene to correct the mutant version. The product of the *p53* gene plays an important role in the response of cells to ionizing radiation, determining whether a cell stops progression in the cell cycle or undergoes apoptosis. Tests of the hypothesis that replacement of p53 in mutant cells increases radiation sensitivity showed that radiation enhances cell killing in human tumor cells that have restored p53 function in vitro and in vivo. Other tumor suppressor genes that may be exploited similarly include *p16* and *p21*, whose products inhibit progression through the G_1 phase of the cell cycle following DNA damage.

At the University of Chicago, Ralph Weichselbaum and colleagues have shown that combining tumor necrosis factor α and radiation enhances tumor cell killing. Because systemic delivery of TNF-α is toxic, the strategy of these investigators was to express the TNF-α gene under the control of a promoter (EGR-1) that is induced by radiation. Irradiated cells with the TNF-α gene under the control of the EGR-1 promoter produce TNF-α, leading to enhanced tumor cell killing without systemic toxicity. This novel approach shows that gene therapy and radiation therapy can be combined effectively.

We developed another strategy combining gene therapy and radiation therapy using adenoviral vectors encoding the DNA for cell-surface receptors to induce high expression levels of the receptors on tumor cells and then treating these cells with high-affinity radiolabeled peptides. Such peptides may overcome the bone marrow toxicity associated with radioimmunotherapy because a low-molecular-weight peptide should be cleared more rapidly from the blood than a radio-

"Gene Therapy" is edited by Joanne T. Douglas and David T. Curiel of the Gene Therapy Program, University of Alabama at Birmingham.



labeled antibody. A peptide should also penetrate a tumor more rapidly than antibody does, leading to a more homogeneous distribution of radioactivity throughout the tumor.

An adenovirus encoding the complementary DNA for human somatostatin receptor substype 2 (SSTr2) was used to infect human ovarian cancer cells in vitro. A significantly greater amount of a peptide labeled with indium-111 bound to SSTr2 on infected cells than on uninfected cells.

For therapy studies, the isotope label must be changed from indium-111, which is an imaging isotope, to a therapeutic isotope. Researchers at Novartis in Basel have shown that a peptide labeled with yttrium-90, which emits β particles, induced complete remissions in rats bearing SSTr2-positive pancreatic tumors. Investigators at Washington University showed tumor growth inhibition in the same animal model using a peptide labeled with copper-64, another β emitter.

Using these therapeutic peptides with our receptor gene transfer strategy should result in increased therapeutic responses in tumors that do not naturally express SSTr2 or express the receptor at a low level. Combining systemic radiation therapy using radiolabeled peptides with gene therapy to express cell-surface receptors may lead to enhanced therapeutic responses in patients with neoplastic disease.

Molecular chemotherapy involves insertion and expression of a gene in a tumor cell and subsequent treatment with a prodrug that acts as the substrate for the gene product, which mediates conversion of the nontoxic prodrug into a toxic drug. The molecular chemotherapy system most widely investigated uses the herpes simplex virus thymidine kinase gene (HSV-TK). Tumor cells expressing HSV-TK are selectively sensitized to the prodrug ganciclovir. After the HSV-TK enzyme phosphorylates the prodrug, cellular kinases metabolize the drug into a nucleoside analog that is incorporated into cellular DNA.

An advantage of this system is a "bystander effect," in which tumor cells not expressing the HSV-TK gene are also killed by the toxic drug produced. It has been postulated that the incorporation of toxic nucleoside analogues into DNA inhibits essential mechanisms related to the function of DNA repair mechanisms, enhancing the radiation sensitivity of that cell. Investigators at Henry Ford Hospital have demonstrated the utility of combining HSV-TK gene therapy and radiation therapy in both in vitro and in vivo models of human cancer.

Other genes have been used in combined molecular chemotherapy and radiation therapy strategies. The *E. coli* cytosine deaminase gene converts the nontoxic prodrug 5-fluorocytosine to 5-fluorouracil. Besides a bystander killing effect, an advantage of this system is the extensive clinical experience with 5-FU as a chemotherapeutic agent and a radiosensitizer.

We evaluated the efficacy of this treatment combination in animal models of gastrointestinal cancer. Adenoviral transfer of cytosine deaminase, systemic 5-FC, and radiation to human cholangiocarcinoma and colon cancer xenografts in athymic nude mice significantly inhibited tumor growth compared to gene therapy and 5-FC without radiation or to radiation therapy alone.

Recently, investigators at Henry Ford Hospital have produced a fusion gene combining HSV-TK and *E. coli* cytosine deaminase, transduced human cancer cells with it, and reported enhanced radiation-induced cytotoxicity with exposure to 5-FC and ganciclovir as compared to exposure to either prodrug alone.

The combination of gene therapy and radiation therapy holds great promise to improve treatment of human malignancies. Future clinical trials will determine the efficacy of these combinations.

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