on local delivery of a prodrug or a suicide gene that would increase sensitivity of tumor cells to cytotoxic drugs. A frequently used strategy has been intratumoral injection of an adenoviral vector expressing the thymidine kinase (*TK*) gene. Cells that take up and express the *TK* gene can be killed after the administration of ganciclovir, which is phosphorylated to a toxic nucleoside by *TK*. Because cell division is required for the toxic nucleoside to affect cell viability, this strategy was initially used in aggressive brain tumors (glioblastoma multiforme) where the cycling tumor cells were affected but the nondividing normal neurons were not. More recently, this approach has been explored for locally recurrent prostate, breast, and colon tumors, among others.

Another local approach uses adenoviral-mediated expression of the tumor suppressor p53, which is mutated in a wide variety of cancers. This strategy has resulted in complete and partial responses in squamous cell carcinoma of the head and neck, esophageal cancer, and non-small-cell lung cancer after direct intratumoral injection of the vector. Response rates (~15%) are comparable to those of other single agents. The use of oncolytic viruses that selectively replicate in tumor cells but not in normal cells has also shown promise in squamous cell carcinoma of the head and neck and in other solid tumors. This approach is based on the observation that deletion of certain viral genes abolishes their ability to replicate in normal cells but not in tumor cells. An advantage of this strategy is that the replicating vector can proliferate and spread within the tumor, facilitating eventual tumor clearance. However, physical limitations to viral spread, including fibrosis, intermixed normal cells, basement membranes, and necrotic areas within the tumor, may limit clinical efficacy. Oncolytic viruses are licensed and available in some countries but not in the United States.

SYSTEMIC APPROACHES

Because metastatic disease rather than uncontrolled growth of the primary tumor is the source of mortality for most cancers, there has been considerable interest in developing systemic gene therapy approaches. One strategy has been to promote more efficient recognition of tumor cells by the immune system. Approaches have included transduction of tumor cells with immune-enhancing genes encoding cytokines, chemokines, or co-stimulatory molecules; and ex vivo manipulation of dendritic cells to enhance the presentation of tumor antigens. Recently, considerable success has been achieved using lentiviral transduction of autologous lymphocytes with a cDNA encoding a chimeric antigen receptor (CAR). The CAR moiety consists of a tumor antigen-binding domain (e.g., an antibody to the B cell antigen CD19) fused to an intracellular signaling domain that allows T cell activation. The transduced lymphocytes can then recognize and destroy cells bearing the antigen. This CAR–T cell approach has proven extraordinarily successful in the setting of refractory chronic lymphocytic leukemia and pre-B-cell acute lymphoblastic leukemia. Infusion of gene-modified T cells engineered to recognize the B cell antigen CD19 has resulted in >1000-fold expansion in vivo, trafficking of the T cells to the bone marrow, and complete remission in a subset of patients who had failed multiple chemotherapy regimens. The cells persist as memory CAR+ T cells, providing ongoing antitumor functionality. Some patients experience a delayed tumor lysis syndrome requiring intensive medical management. This approach also causes an on-target toxicity, leading to B cell aplasia that necessitates lifelong IgG infusions. Current results indicate that long-lasting remissions can be achieved and the strategy can theoretically be extended to other tumor types if a tumor antigen can be identified.

Gene transfer strategies have also been developed for inhibiting tumor angiogenesis. These have included constitutive expression of angiogenesis inhibitors such as angiostatin and endostatin; use of siRNA to reduce levels of VEGF or VEGF receptor; and combined approaches in which autologous T cells are genetically modified to recognize antigens specific to tumor vasculature. These studies are still in early-phase testing.

Another novel systemic approach is the use of gene transfer to protect normal cells from the toxicities of chemotherapy. The most extensively studied of these approaches has been transduction of hematopoietic cells with genes encoding resistance to chemotherapeutic agents, including the multidrug resistance gene *MDRI* or the gene encoding O^6-methylguanine DNA methyltransferase (*MGMT*). Ex vivo transduction of hematopoietic cells, followed by autologous transplantation, is being investigated as a strategy for allowing administration of higher doses of chemotherapy than would otherwise be tolerated.

GENE THERAPY FOR VASCULAR DISEASE

The third major category addressed by gene transfer studies is cardiovascular disease. Initial experience was in trials designed to increase blood flow to either skeletal (critical limb ischemia) or cardiac muscle (angina/myocardial ischemia). First-line treatment for both of these groups includes mechanical revascularization or medical management, but a subset of patients are not candidates for or fail these approaches. These patients formed the first cohorts for evaluation of gene transfer to achieve therapeutic angiogenesis. The major transgene used has been VEGF, attractive because of its specificity for endothelial cells; other transgenes have included fibroblast growth factor (FGF) and hypoxia-inducible factor 1, α subunit (HIF-1α). The design of most of the trials has included direct IM (or myocardial) injection of either a plasmid or an adenoviral vector expressing the transgene. Both of these vectors are likely to result in only short-term expression of VEGF, which may be adequate because there is no need for continued transgene expression once the new vessels have formed. Direct injection favors local expression, which should help to avoid systemic effects such as retinal neovascularization or new vessel formation in a nascent tumor. Initial trials of adeno-VEGF or plasmid-VEGF injection resulted in improvement over baseline in angiographically detectable vasculature, but no change in amputation frequency or cardiovascular mortality. Studies using different routes of administration or different transgenes are currently under way.

More recent studies have used AAV vectors to develop a therapeutic approach for individuals with refractory congestive heart failure. In preclinical studies, a vector encoding sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a) demonstrated positive left ventricular inotropic effects in a swine model of volume-overloaded heart failure. Results of a phase II study in which vector was infused via the coronary arteries in patients with congestive heart failure demonstrated safety and some indications of efficacy; larger studies are now planned.

OTHER APPROACHES

This chapter has focused on gene addition therapy, in which a normal gene is transferred to a target tissue to drive expression of a gene product with therapeutic effects. Another powerful technique under development is genome editing, in which a mutation is corrected in situ, generating a wild-type copy under the control of the endogenous regulatory signals. This approach makes use of novel reagents including zinc finger nucleases, TALENs and CRISPR, which introduce double-stranded breaks into the DNA near the site of the mutation and then rely on a donated repair sequence and cellular mechanisms for repair of double-strand breaks to reconstitute a functioning gene. Another strategy recently introduced into clinical trials is the use of siRNAs or short hairpin RNAs as transgenes to knock down expression of deleterious genes (e.g., mutant huntingtin in Huntington's disease or genes of the hepatitis C genome in infected individuals).

SUMMARY

The power and versatility of gene transfer approaches are such that there are few serious disease entities for which gene transfer therapies are *not* under development. The development of new classes of therapeutics typically takes two to three decades; monoclonal antibodies and recombinant proteins are recent examples. Gene therapeutics, which entered clinical testing in the early 1990s, traversed the same time course. Examples of clinical success are now abundant, and gene therapy approaches are likely to become increasingly important as a