Meeting Report

Gene Therapy and Translational Cancer Research

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The Pathology B and Experimental Therapeutics-2 study sections sponsored a workshop devoted to recent advances in gene therapy and translational research relevant to cancer. The workshop was highlighted by a historical perspective of gene therapy by Dr. Theodore Friedman (University of California, San Diego, CA). The opening remarks that human gene therapy is not a current success set the tone for a rigorous examination of this approach to cancer treatment. There are currently approximately 300 approved gene therapy trials, 188 of which involve cancer biology or therapy. Gene therapy is defined as the ability to treat disease at the level of the underlying gene defect, whether it is applied to cure genetic diseases, target somatic cells with single gene defect, or treat complex diseases or nongenetic diseases such as AIDS. The obstacles surrounding effective human gene therapy have been studied by the Orkin-Motulsky Committee commissioned by Dr. Harold Varmus, director of the NIH (Bethesda, MD). This committee found human gene therapy to be an immature science with limited understanding of gene regulation and disease models for preclinical studies. Recommendations are to set high standards for clinical studies, encourage and support interdisciplinary studies, and focus on basic issues of molecular virology. This workshop reviewed some recent advances in gene delivery, gene expression, immune manipulation, and the development of molecular targets and stressed that all of these fields will need further advancement to make gene therapy a reality.

As an example of the promise and pitfalls of gene therapy, the pioneering work of Anderson and Blaese was reviewed by Dr. Theodore Friedman, and recent studies were reviewed by Dr. Donald Kohn (Children’s Hospital Los Angeles, University of Southern California School of Medicine, Los Angeles, CA). Although the transduction of the ADA3 gene into the lymphocytes of patients afflicted by immunodeficiency by Anderson and Blaese was accomplished, the data indicate that no therapeutic effect has been gained from this initial study. In a clinical trial started in 1993, three infants with ADA-deficient SCID were given autologous umbilical cord blood CD34+ (CD34 is a marker associated with hematopoietic stem) cells transduced with normal human ADA cDNA. The patients were also treated weekly with i.m. PEG-ADA. Four years later, they continue to produce leukocytes containing ADA cDNA, with approximately 100-fold higher frequencies of gene-containing T lymphocytes (1–10%) than other lineages (0.01–0.1%). In the summer of 1997, the investigators stopped the PEG-ADA replacement for one patient; multiple laboratory and clinical parameters of immune function declined during the 2-month period, and PEG-ADA treatment was reinstituted.

This scientific “proof of principle” but clinical defeat clearly showed that more work was necessary on vector engineering for greater transduction efficiency and gene expression. As such, work to improve vectors for gene delivery has recently offered some promises. These include the improvement of retroviral vectors through pseudotyping. That is, by engineering the VSV-G glycoprotein on the surface of retroviral particles, retroviruses are able to effectively enter the lipid membrane of cells through an unknown mechanism. Other recent advances in the development of improved viral vectors were discussed by Dr. Inder Verma (The Salk Institute, La Jolla, CA), who reviewed some of the evolution of gene therapy from the perspective of the effort to correct the coagulation factor IX deficiency as a gene therapeutic model. Initial studies with retroviral vectors expressing factor IX in fibroblasts resulted in only short-term expression. Expression in myoblasts was achieved through the use of muscle-specific creatine kinase enhancer promoter sequences, with high levels of factor IX production lasting for up to 2 years when transduced myoblasts were implanted into nude mice. However, when the same approach was taken with factor IX-deficient dogs, the dog myoblasts transduced with factor IX-expressing adenoviruses ceased to produce factor IX after 20 days in vivo. It became readily apparent that the immune-competent animals mounted a brisk antibody and cellular immune response to the viruses and transduced cells, thereby eliminating the expression of factor IX. Other models using adenoviral vectors have succumbed to similar limitations by the host immune response to adenoviruses. Thus, new vectors are needed to circumvent these limitations.

An ideal vector is expected to: (a) be available at high titers; (b) be convenient; (c) be reproducible; and (d) confer no immune responses. The lentiviruses, such as HIV, have been exploited to provide these characteristics. In particular, Dr. Verma described a vector producing viral particles in which the surface gp120 of HIV has been replaced by the VSV-G protein that allows entry into cells. The HIV-based vectors are able to transduce genes into cells independent of their growth status. Recent studies have demonstrated that the HIV-based vectors are capable of conferring the ectopic expression of genes in neuronal cells of the central nervous system, and there has been...
no evidence of brisk immune responses to the HIV-based vectors.

Some of the problems of high efficiency transfection might become less important if the introduced gene were in a renewable stem cell, or if its expression conferred a survival benefit. Dr. Kenneth Cowan (Medicine Branch, National Cancer Institute, Bethesda, MD) discussed the hematopoietic reconstitution of CD34-selected cells transduced with the multidrug resistant MDR1 gene in patients with metastatic breast cancer as a model of hematopoietic stem cell marking and drug tolerance. His group has conducted two clinical trials of retroviral-mediated transfer of the multidrug resistance gene MDR1 into PBPCs in patients with metastatic breast cancer to determine whether chemotherapy after reinfusion of transduced cells can selectively expand the population of MDR1-marked hematopoietic cells. In the first trial, patients were treated with high-dose ifosfamide, carboplatin, and epirubicin chemotherapy along with hematopoietic stem cell support. In this trial, only one-third of the harvested CD34+ cells were transduced with a retroviral vector expressing the MDR1 gene. After reconstitution, all patients were treated with five cycles of paclitaxel, and the level of MDR1 gene marking in the peripheral blood was analyzed after each cycle of therapy. Three of the four patients had detectable MDR1 gene marking at reconstitution, and two patients had MDR1 marking of granulocytes throughout the five cycles of therapy.

The Cowan group then initiated a second trial in which patients received only CD34+ cells exposed to retroviral gene transduction conditions to avoid the potential competition for engraftment with nontransduced cells. Six patients received genetically altered CD34+ PBPCs; half of the cells were incubated in supernatant from a retroviral producer clone containing the MDR1 gene, and the other half were incubated in supernatant from a retroviral producer clone containing the neomycin resistance (NeoR) gene. A maximum of 1.5 × 10⁶ CD34+ selected cells/kg were transduced with each vector and transfused. In two of six patients, both peripheral granulocyte and monocyte fractions showed no detectable marking early in the course of treatment after reconstitution but later demonstrated evidence of in vivo selection of cells containing MDR1; there was no selection of cells containing NeoR. In the remaining four patients, early marking was apparent, but long-term expansion with chemotherapy was not observed. The difference in long-term granulocyte and monocyte marking by MDR1 compared to NeoR in two of six patients suggests MDR1-transduced stem cells were selected in vivo by chemotherapy in these patients. This study also demonstrated the safety of using only PBPCs incubated in retroviral supernatant for hematopoietic reconstitution because all six gene therapy patients recovered from myelosuppressive therapy, and each went on to receive eight cycles of intensive chemotherapy.

A similar strategy to genetically alter drug metabolism in hematopoietic cells was taken by Dr. James Doroshow (City of Hope National Medical Center, Duarte, CA). Chemoprotective strategies involve the use of bicistronic vectors capable of expressing genes encoding glutathione peroxidase or glutathione S-transferase. Ectopic expression of glutathione peroxidase confers tolerance to high levels of colchicine and protects cells against oxidative stress, for example, with glucose oxidase exposure. Transduction of the MDR1 gene was also undertaken, and selection for the MDR1 gene was enhanced by the exposure of cells to colchicine. In fact, colchicine was more effective in the selection of MDR1-expressing cells than G418 selection. When applied to mouse and human CD34+ cells, however, the transduction efficiency of MDR1 was very low (about 20%). Alternative gene transfer strategies such as lipofection appear to be feasible in human CD34+ cells, resulting in up to 50% marked cells. Thus, the limitations of efficient gene transfer require additional studies of alternative delivery options, such as lipofection, or the development of better vectors.

Even with the efficient delivery of genes and a way to enrich for cells expressing those gene products, pitfalls remain in stable gene expression. Dr. Donald Kohn discussed retroviral vectors with multiple modifications in the cis-elements implicated in transcriptional silencing. These modified vectors showed a >1000-fold higher expression of NeoR or chloramphenicol acetyltransferase reporter gene than did standard MoMuLV LTR vectors in murine ES cells. The modified MND LTR directed expression of the E-GFP reporter in essentially 100% of transduced ES cells, whereas unmodified MoMuLV LTR was active in only 1–5% of transduced ES cells. The modified vectors have now been evaluated in the serial murine bone marrow transplantation model and were found to be significantly less methylated than MoMuLV LTR. The modified vectors were expressed in >90% of hematopoietic colony-forming units, whereas MoMuLV LTR was expressed in only 10–20% of hematopoietic colony-forming units. The MND vector also showed a higher level of expression in T and B lymphocytes produced in the mice transplanted with transduced bone marrow. Lenti vectors are currently being examined to see if they will increase the transduction of primitive pluripotent human hematopoietic stem cells.

In the long term, the application of gene therapy methods is limited by our knowledge of how genes are silenced or regulated within a chromosomal environment. Dr. Robert Oshima (The Burnham Institute, La Jolla, CA) presented recent findings regarding the transcriptional regulation of transgenes and sequences that regulate chromatin structure and gene expression. Many or most genes integrated into cells and animals are subject to position effects that alter the expression of the gene. The Oshima laboratory has discovered cis-acting sequences flanking the keratin 18 gene (K18) that confer integration site-independent and copy number-dependent expression of both the K18 gene and two tested heterologous genes in transgenic mice. They have found that the smallest K18-flanking fragment able to confer position-independent expression on the metallothionein-human growth hormone transgene is a 340-bp fragment composed primarily of an Alu-repetitive element. This element, which is transcribed by RNA polymerase III, was found to protect the transgene in an orientation-dependent manner, with the most effective arrangement consisting of inverted repeats flanking the transgene. Furthermore, when interposed between a synthetic telomere that nucleates repressive chromatin structure and a URA3 reporter gene, the Alu sequence was able to block chromatin-mediated repression in yeast. In a genetic background that relieved chromatin-mediated repression due to the absence of the Sir3 gene product, the Alu sequence had no effect. This confirmed that the activity of the Alu was due to blocking chromatin-mediated repression and not simply to elevating the transcriptional activity of the URA3 gene. This effect, like the protection afforded in transgenic mice, was
dependent on the orientation of the Alu sequence. These results indicate that much of the locus control region, like the activity of the K18-flanking sequences, is associated with a small 340-bp Alu sequence, and that the same element was active in yeast to block chromatin-mediated repression. cis-acting elements like the K18 Alu fragment may facilitate the expression of other genes when integrated into random locations within the genome. It also suggests that a subset of Alu sequences may function to define regulatory domains within the human genome and leads to speculation about the function of Alu elements in gene regulation and during evolution.

Just as knowledge of the basic biology surrounding viral transfection and gene expression will advance translational achievements in gene therapy, a better knowledge of the immune system is furthering its potential application toward cancer vaccine therapy. Dr. James Economou (University of California Los Angeles School of Medicine, Los Angeles, CA) discussed the use of genetically engineered dendritic cell animal models. Dendritic cells are professional antigen-presenting cells that process antigens and present them to T cells as part of a complex reaction leading either to tolerance or to CTL-mediated elimination of noncognate cellular targets. As a model, the melanoma MART-1 peptide antigen was used to engineer dendritic cells that express MART-1. CTLs readily recognize dendritic cell clones transduced with MART-1 expression vector. The tumor model is a transplanted MART-1-expressing murine fibrosarcoma that is tumorigenic but is normally poorly immunogenic. Murine bone marrow cells were treated with GM-CSF and interleukin 4 before being harvested for dendritic cells, which were transduced with the MART-1 adenoviral expression vector and injected into tumor-bearing animals. The dendritic cell-treated animals demonstrated a marked suppression of tumor growth as compared to control-treated animals. In 20% of animals, no tumor growth was detected, whereas the remaining 80% demonstrated a delayed outgrowth after treatment with engineered dendritic cells. Curiously, repeated immunizations worsened the outcome that correlated with a decrease in the CTL assay. In addition, both CD4 and CD8 cells were necessary for protection against tumor formation. Clinical trials are ongoing with adenovirus MART-1-transduced cells. As additional proof of principle, the α-fetoprotein was exploited in the immune-mediated killing of hepatocellular carcinoma cells. With the BWIC3 hepatoma cell line as a tumor model, immunization with adenovirally transduced AFP dendritic cells resulted in a marked protection against tumor formation. Additional studies revealed two dominant AFP peptides presented by HLA-2 molecules that play a role in CTL response. Through specific peptide loading experiments, it was demonstrated that specific lysis of AFP-expressing cells can be elicited. These studies suggest that engineered dendritic cells and specific immunogenic peptide loading may provide additional means to attack cancer cells.

Dr. Edmund C. Lattice (Thomas Jefferson University, Philadelphia, PA) discussed the use of vaccinia virus for immunotherapy. The principal goal of immunotherapeutic strategies in tumors is the induction of a systemic cell-mediated antitumor response that would eliminate both primary and metastatic tumor foci. Applying earlier findings by Pardoll and coworkers, the Lattice group has proposed an approach to immunological gene therapy in which the genes for immune-enhancing cytokines and cell surface antigens can be transduced into tumor cells in situ using vaccinia virus vectors. Lattice has evaluated recombinant vaccinia encoding GM-CSF as a candidate molecule to enhance the generation of tumor-specific immunity by enhancing antigen presentation. In murine studies, they show that the GM-CSF gene is expressed both early and late in the course of therapy, and that preexisting or developing immunity to vaccinia does not prevent GM-CSF gene expression. In a Phase I clinical study of intraliesional recombinant vaccinia-GM-CSF in s.c. melanoma metastases, they found that the recombinant can be given safely, and the GM-CSF gene is expressed both early and late in the treatment course. Intraliesional injection of the recombinant results in the regression of injected lesions, which is most probably due to the antivaccinia inflammatory response. In addition, in a number of patients, they have found that distant unjected lesions regress, suggesting the development of systemic antitumor immunity. These studies support the use of vaccinia recombinants for in situ use as vectors for inserting immune active molecules into tumor cells.

Dr. Alfred E. Chang (University of Michigan, Ann Arbor, MI) discussed the applications of gene transfer in the adoptive immunotherapy of cancer. Adoptive immunotherapy of cancer involves the passive transfer of immune cells that are capable of conferring systemic immunity to the host to mediate tumor regression. Traditionally, this has involved the isolation and expansion of T cells from either TILs or tumor-draining lymph nodes. With the advent of gene transfer methodologies, new techniques to enhance the generation of immune T cells have been explored. TILs represent lymphoid cells derived from tumors that are disaggregated ex vivo and cultured in interleukin 2. A significant impediment to generating therapeutic TIL resides in the inherent immunogenicity of the tumor from which the TILs are derived. In animal models using poorly immunogenic tumors, the therapeutic efficacy of the TILs is limited. A novel variation to altered TIL reactivity has been developed at the University of Michigan. In animal studies, tumors treated by the in vivo transfer of an allogeneic MHC class I gene complexed with liposomes resulted in the expression of the class I molecules by tumor cells. Importantly, this also resulted in tumor regression and the induction of T cells that were not only reactive to transfected tumor cells, but also to unmodified cancer cells. They have previously reported evidence of gene expression in advanced melanoma patients treated by in vivo inoculation of the tumor with DNA/liposome complexes containing a foreign MHC class I gene. Based on these initial observations, they are currently evaluating the immune reactivity of TILs derived from tumors modified by direct in vivo gene transfer using an allogeneic class I MHC gene, HLA-B7, in patients with stage IV melanoma. In in vitro assays, they have confirmed an enhanced reactivity of TILs derived from patients inoculated with the foreign class I gene. Another method of T-cell therapy has been to activate tumor-draining lymph node cells, which they have demonstrated to have potent antitumor reactivity on adoptive transfer in preclinical studies. In a poorly immunogenic tumor model, they have found that genetically modifying tumor cells to secrete GM-CSF results in significant tumor reactivity of the tumor-draining lymph node cells. They hypothesize that the local secretion of GM-CSF promotes the recruitment and expansion of antigen-presenting dendritic cells. This approach is being evaluated in a clinical trial at the University of Michigan in which patients with stage IV melanoma are vaccinated with autologous melanoma tumor cells transduced to secrete GM-CSF. Thus far, the vaccine sites have demonstrated a local influx of
dendritic cells. They have been able to define autologous tumor cell reactivity of the vaccine-primed lymph node cells. One patient with metastatic disease treated with these adoptively transferred cells has experienced a complete response. They feel that the use of gene transfer techniques may result in more effective methods to generate tumor-specific T cells for cellular therapy.

Even in the perfect scenario, in which all of the problems with gene delivery and expression are solved, these technologies may be fruitless without a good understanding of the basic defects in cancer biology and the correct choices of molecular targets for gene therapy. A part of this workshop revolved around the more basic issues of the use of alternative cancer gene therapy approaches and translational research applied to molecular profiling and anticancer drug discovery. Dr. Ming-Chie Hung (The University of Texas M. D. Anderson Cancer, Houston, TX) took a novel approach toward cancer gene therapy by exploiting the tumor suppression activity of E1A in HER-2/neu-overexpressing cancer cells. The HER-2/neu (also named c-erbB-2) oncogene is known to be overexpressed in many human cancers, including breast, ovarian, lung, gastric, and oral cancers. In animal models, HER-2/neu overexpression was shown to enhance malignancy and metastasis phenotypes. Repression of HER-2/neu overexpression suppresses the malignant phenotypes of HER-2/neu-overexpressing cancer cells, suggesting that HER-2/neu may serve as an excellent target for developing anticancer agents. The Hung group has previously shown that adenovirus-5 E1A gene products inhibit overexpression of the HER-2/neu oncogene and accordingly suppress transformation induced by HER-2/neu. Their results indicate that cationic liposomes or an adenoviral vector can efficiently deliver E1A into tumor cells in mice, and this results in the suppression of tumor growth and longer survival of the mice. Based on these results, a Phase I gene therapy clinical trial entitled “Phase I Study of E1A Gene Therapy for Patients with Metastatic Breast or Ovarian Cancer that Overexpresses HER-2/neu” was initiated at M. D. Anderson Cancer Center (P. I. Gabriel Hortobagyi). The data from the clinical trial indicate that the E1A gene can be expressed in treated patients, and HER-2/neu down-regulation in tumor cells is readily detectable. A maximum tolerable dose is also identified. The result suggests that E1A-liposome gene therapy is feasible, and additional clinical trials on patients with less-malignant cancer are required to evaluate therapeutic efficacy.

Dr. Patricia Steeg (National Cancer Institute) discussed Nm23 as a potential therapeutic target. Nm23 expression is reduced in metastatic cells, and the introduction of Nm23 into certain cancer cell lines has reduced their metastatic potential. One approach to the problem of metastasis is to up-regulate the expression of Nm23. As such, Steeg and coworkers studied the Nm23 promoter and found three breast-specific elements consisting of a consensus 5'-ACAAAG-3' site and NF1 and ETS binding sites. Although the regulation of Nm23 expression has not been exploited for therapeutic purposes, the Steeg group has used the COMPARE computer algorithm and Nm23 expression as a marker of tumor metastatic potential to examine the in vitro antiproliferative activity of chemotherapeutic drugs on human breast carcinoma and melanoma cell lines. None of the compounds tested were effective in vitro, and only 40 of 30,000 repository compounds exhibited preferential growth inhibition of low-Nm23-expressing metastatically aggressive cell lines with a Pearson correlation coefficient of \( r \leq -0.64 \). Characterization of one compound, NSC 645306, is presented including in vivo activity in a hollow fiber assay. The data demonstrate a novel approach using molecular markers and the NCI 60 human cancer cell line panel to identify potential drugs for aggressive human tumors.

Dr. Chi Van Dang (Johns Hopkins University School of Medicine, Baltimore, MD) presented recent studies on c-Myc target genes and their therapeutic implications. Based on the frequency of genetic alterations of c-Myc in human cancers, it can be estimated that approximately 70,000 United States cancer deaths per year are associated with changes in the c-myc gene or its expression. Given that c-myc may contribute to one-seventh of United States cancer deaths, the understanding of c-myc is important to our intellectual armamentarium against cancer. The c-Myc gene encodes an oncogeni helix-loop-helix-leucine zipper transcription factor that acts as a heterodimer with its partner protein, Max, to activate genes regulating cell growth, differentiation, and cell death. To further understand c-Myc function, the Dang group has identified a set of putative c-Myc-responsive genes through the application of cDNA representation difference analysis. They identified 17 up-regulated genes and 3 down-regulated genes in a rat fibroblast system that is susceptible to c-Myc-mediated transformation. Two novel genes, rcl and JPl, cause anchorage-independent growth, although at a very reduced efficiency compared to c-Myc. Three other genes, KAP1, Mer5, and LDH-A (encoding lactate dehydrogenase A) are nontransforming; however, LDH-A is required for the establishment of three-dimensional spheroid growth in soft agar. Furthermore, LDH-A participates in c-Myc-mediated glucose deprivation-induced apoptosis. This observation is being exploited for therapeutic purposes. However, in addition to the study of target genes, Dang and coworkers observed that over-expression of c-myc causes Colcemid-treated human and rodent cells to replicate DNA without chromosomal segregation and become either apoptotic or polyploid. c-Myc protein levels were further correlated with the sensitivity of 60 human cancer cell lines to tubulin binding drugs, suggesting that this novel phenotype of c-Myc sensitizes cells to this class of antitumor drug.

This approach toward studying the genetic program enforced by c-Myc in cellular transformation has led to the identification of novel connections between cellular metabolism and transformation as well as the identification of novel genes that probably account for some of the transforming activities of c-Myc.

The studies presented at this workshop provide a glimpse of the past and present and a projection of the future of cancer gene therapies and novel therapeutic approaches based on sound molecular underpinnings. Although significant progress has been achieved in our understanding of the limitations of gene therapy by suboptimal vectors, host immunological responses to the vectors, and the lack of long term stable expression, the major challenge that limits clinical translation remains in achieving efficient gene delivery to target tissues. As such, gene therapy that exploits the host immune system and the development of novel small molecular therapeutics might hold promise for the future. Whereas setbacks in gene therapy were clearly recognized and discussed, there was a unique level of enthusiasm that many of these obstacles could be overcome with meticulously designed basic and clinical studies.