Advances in Gene Therapy for Malignant Melanoma

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Background: The recent developments in the field of gene transfer have advanced the use of gene therapy as a novel strategy against a variety of human malignancies. Due to its unique set of characteristics, melanoma represents a suitable target for the clinical translation of the different gene transfer approaches recently developed. The goal of gene therapy targeted to melanoma cells is to introduce “suicide” genes, to transfer tumor suppressor genes, to inactivate aberrant oncogene expression, or to introduce genes encoding immunologically relevant molecules. Gene therapy targeted to the host’s immune cells has been developed as an additional strategy to redirect immune responses against melanoma.

Methods: The authors reviewed the published gene transfer studies in experimental models, as well as the results of gene therapy clinical trials for patients with melanoma.

Results: Clinical trials have shown the feasibility and safety of gene therapy against malignant melanoma. Although no major successes have been reported, the positive results observed in some patients support the potential for gene therapy in the management of this disease.

Conclusions: Gene therapy of melanoma using current gene transfer approaches is feasible and safe. Better vector technology as well as increased understanding of the “bystander effect” triggered by gene transfer approaches would provide the tools to validate gene therapy as an effective modality of treatment for malignant melanoma.
Introduction

The past several years has witnessed an impressive improvement in the efficacy of conventional therapeutic strategies against cancer. More sophisticated and less invasive surgical procedures, improved radiation delivery, and new, more potent chemotherapeutic agents have led to increased responses and even cures in a variety of tumor types. Despite this significant progress, the treatment of malignant melanoma, especially metastatic disease has continued to challenge physicians, with only modest improvement in response rates following conventional anticancer treatment.1

In recent years, efforts have focused on the identification of novel non-cross-resistant modalities of treatment that could potentially improve the management of metastatic melanoma. In spite of its intrinsic resistance to treatments currently available, melanoma offers a unique set of characteristics that make it a suitable target for new therapeutic strategies. Indeed, the recent advances in our understanding of the genetic and molecular abnormalities underlying the progression of malignant melanoma,2,3 the identification of melanoma specific tumor antigens,4 and the easy accessibility to tumor lesions have brought to the clinical trial arena the use of gene therapy as a promising strategy against this disease.

Management of Malignant Melanoma: Developments in Gene Therapy

In gene therapy of melanoma, foreign genes are introduced into either tumor cells or the host’s immune cells (Table). The goal of gene therapy targeted to melanoma cells is (1) to introduce “suicide” genes, (2) to transfer tumor suppressor genes, (3) to inactivate aberrant oncogene expression, or (4) to introduce genes encoding immunologically relevant molecules such as co-stimulatory molecules and/or cytokines. Gene therapy targeted to the host’s immune cells, ie, melanoma-infiltrating lymphocytes or dendritic cells, has been developed as an additional strategy to redirect immune responses against melanoma.

Despite the initial enthusiasm generated by successful gene transfer in preclinical models, the field of gene therapy of human melanoma is still in its infancy. While no major successes have been reported in recently completed clinical trials, the limited positive results obtained have shown the feasibility and safety of this approach. More importantly, these early clinical studies have unveiled the limitations and obstacles that would need to be overcome to make gene therapy an effective treatment modality for malignant melanoma.

In this regard, the developments that have occurred in recent years — better vector technology transfer as well as an increased understanding of the cellular and molecular mechanism involved in the so-called “bystander effect” elicited by gene therapy — indicate that this approach may become efficacious in the foreseeable future.

Current Limitations of Gene Therapy Strategies

In the early days of gene therapy, the successful delivery of foreign genes into murine cells by retroviral vectors raised excitement over the feasibility of translating these findings into the treatment of certain genetic human diseases, including cancer. However, despite the significant progress achieved in vector technology as well as in the in vivo and ex vivo delivery of genes into murine experimental tumors,5 studies of gene transfer in human subjects have shown that an effective gene therapy of cancer remains elusive.

One important limitation of the current generation of gene therapy strategies is that vector technology has not yet progressed to the point of specifically targeting tumor cells following systemic administration of the vector carrying the gene of interest. The need for selective transduction of tumor cells to avoid the transfection of normal cells is critical in order to minimize toxicity. Furthermore, the ability to target tumor cells in multiple sites is extremely important if the goal is to
control metastatic disease, which is the main cause of treatment failure and death in melanoma patients. In the absence of such a vector, most clinical trials have relied on gene delivery directly into accessible tumors.

An additional problem of this technology is the efficiency of gene transfer, particularly when used for the inactivation of oncogenes, the replacement of tumor suppressor genes, or the introduction of suicide genes. With these strategies, it is necessary to deliver the gene to every cell; otherwise, the remaining non-transduced malignant cells will continue to proliferate, leading to disease relapse following an apparent “good initial response.”

Despite these limitations, one of the most interesting observations in the field of gene therapy relates to the so-called bystander effect. Studies with p53 gene and suicide gene transfer strategies showed that in spite of low gene transfer efficiency into tumor cells, the magnitude of the cell killing far exceeded what would be expected based on transfection efficiency. Multiple mechanisms, including cell-cell transfer of toxic substrates, angiogenesis inhibition, and an immune component, have been suggested to play a role in this bystander effect. However, the cellular and molecular mechanisms underlying this effect are not fully elucidated. Further understanding and amplification of the bystander effect may provide a unique opportunity to overcome the limitations imposed by the low transfer efficiency of the vectors currently available.

Introduction of Suicide Genes Into Melanoma Cells

The genes used in the suicide gene transfer strategy are those that, when introduced into tumor cells, have the capacity to convert a nontoxic prodrug into a toxin within the tumor cell. The most widely used gene in clinical trials using this approach is the herpes simplex virus thymidine kinase (HSVtk) gene. This gene is introduced into tumor cells, and patients are subsequently given the drug ganciclovir. This drug is an acyclic nucleoside analogue that, when phosphorylated by HSVtk, is incorporated into DNA (as ganciclovir-triphosphate) resulting in the termination of DNA elongation during S-phase of transduced tumor cells. The human thymidine kinase has a low affinity for ganciclovir and therefore this drug has little toxicity in humans. Using this strategy, a significant antitumor effect was initially demonstrated in a B16 melanoma model and in a xenograft melanoma model. The degree of tumor size reduction (up to 80% reduction of viable tumor) was often disproportionate to the expected degree of transduction efficiency, due to the killing of neighboring untransduced melanoma cells (bystander effect). Further studies of HSVtk gene-mediated cell killing suggest a role for immune-mediated antitumor responses in the observed bystander effect.

Given these results, Klatzmann et al designed a phase I/II dose escalation study of herpes simplex virus type 1 thymidine kinase (HSV-1-tk) suicide gene therapy for patients with metastatic melanoma. In this study, metastatic nodules of eight melanoma patients were directly injected with a murine cell line producing a nonreplicating retroviral vector encoding HSVtk. After a 7-day period, ganciclovir infusions were administered for 14 days. HSV-1-tk gene therapy was well tolerated over a wide dose range, and only mild and transient adverse events such as local inflammatory skin reactions and fever were observed. However, the antitumor effect was limited since the treated tumor size was moderately affected under ganciclovir compared with untreated tumors, and all patients showed disease progression on long-term follow-up. Interestingly, in three of eight patients, significant tumor necrosis (>50%) was observed in nodules injected with the vector suggesting a direct toxic effect of ganciclovir triphosphate. One of the major barriers encountered in this study was the low transfection efficacy (less than 1%), which may explain, at least in part, the limited efficacy of this strategy in humans. To overcome these limitations, the same investigators have developed replicating as well as semireplicating retroviral vectors to achieve transgene expression in transfected cells followed by ulceration and release of the vector that can then infect dividing neighboring cells, thus amplifying the efficiency of this strategy.

Transfer of Tumor Suppressor Genes

The most common gene used in clinical trials utilizing the strategy of replacing defective tumor suppressor genes is the p53 gene. This gene is the most frequently mutated tumor suppressor gene in human cancers. Although point mutations of this gene are rare in melanoma, overexpression of this gene in melanoma cells resulted in apoptosis of not only tumor cells expressing mutated p53, but also those cells containing the wild-type form. Cirielli et al used an adenoviral vector to induce overexpression of wild-type p53 in either murine B16 melanoma or human SK-MEL-24 melanoma cell lines. This strategy resulted in apoptosis of these cells in vitro as well as inhibition of tumor growth in vivo. More recently, Dummer et al evaluated the biological activity and safety of intratumoral injection of a wild type p53 adenoviral vector in five patients with metastatic melanoma and one breast can-
cer patient with increased p53 protein immunoreactivity in pretreatment tumor biopsies. This phase I dose escalation study of a single injection of replication defective adenoviral vector was associated with minimal toxicity. Furthermore, biological activity of the injected wild type p53 was demonstrated in five of the six patients by reverse transcriptase-polymerase chain reaction (RT-PCR) of tumor tissue 2 days after intratumoral injection. Future clinical trials would determine whether this approach either alone or in combination with other therapeutic modalities might have a role in the treatment of patients with melanoma.

Two potential shortcomings may limit the use of adenovirus to express p53 in malignant cells. First, the gene expression is not regulated in tumors, and second, the expression of mutant forms of p53 that can act in a dominant negative fashion are not affected by this gene replacement approach. To overcome this obstacle, ribozymes have been recently used to simultaneously restore wild-type p53 function and reduce the expression of mutant p53 in various human cancers. Ribozymes are RNA molecules with catalytic activity that can cleave RNA.\textsuperscript{21} Watanabe and Sullenger\textsuperscript{22} have recently used a transsplicing group I ribozyme that repairs mutant p53 mRNAs with high fidelity and specificity. These investigators found that the uridines at positions 41 and 65 in the p53 coding sequence are particularly accessible for ribozyme binding and activity. The ribozyme then cleaves the target mRNA and releases the downstream RNA sequence containing the p53 mutation(s) and replaces the sequence with a 3’ exon that encodes the correct sequence for the wild-type transcript. More importantly, the corrected transcripts are successfully translated to functional p53 able to transactive p53-responsive promoters and down-modulate expression of the multidrug resistance (MDR1) gene promoter. This innovative approach, however, still requires novel and more efficient gene transfer systems before it can be translated into the clinical trial arena for the treatment of human malignancies, including melanoma.

In addition to p53, other tumor suppressor proteins are good targets for replacement gene therapy approaches. The control of cellular proliferation and the cell cycle is highly dependent on the G\textsubscript{1} cyclins and a family of proteins termed cyclin-dependent kinases that negatively regulate the function of cyclins. One of the best characterized genes involved in the pathogenesis of melanoma is p16INK4a.\textsuperscript{3} This gene encodes for a cyclin-dependent kinase inhibitor that blocks the kinase activity of CDK4 and CDK6, which in turns prevents pRB phosphorylation and G\textsubscript{1}/S phase progression. Mutations in p16INK4a result in loss of p16 inhibitory function leading to deregulation of the cell cycle and enhanced proliferation of tumor cells. Preclinical studies have demonstrated that replacement of this protein using adenoviral vectors is feasible and could be of benefit in the treatment of certain cancers. Kawabe et al\textsuperscript{23} have recently shown that re-expression of p16INK4a sensitizes cancer cells to radiation treatment in a p53-dependent manner. The clinical application of this strategy in metastatic melanoma remains to be investigated.

### Blockade of Oncogenic Signaling Pathways

In addition to the mutation of tumor suppressor genes, the constitutive activation of oncogenes such as members of the ras family and c-myc frequently occurs in melanoma and contributes to the malignant phenotype.\textsuperscript{3,24} These genes therefore represent additional targets for gene therapy of melanoma. Several potential strategies can be used to reduce the expression of activated oncogenes. One method is to introduce a gene encoding for a ribozyme, which is an RNA that has catalytic activity and cleaves mRNA resulting in reduced expression of products of the oncogene.\textsuperscript{25} Another method of inactivating oncogene mRNAs is to introduce a gene that encodes for the oncogene antisense.\textsuperscript{26} When expressed in tumor cells, the antisense nucleotides block translation by binding to the oncogene mRNA and also target the mRNA for degradation by RNase H. Yet another method would be to express “dominant negative” mutant forms of the oncogene that either bind the oncogene or bind to downstream effector molecules and prevent activation of the involved signaling pathway.\textsuperscript{27} One final method of inactivating oncogenes is to introduce the gene that encodes for a portion of an antibody molecule, referred to as a single-chain Fv molecule (scFv), that is specific for the oncogene product. When expressed within the tumor cells, the scFv can bind to and thereby inactivate the oncogene product.\textsuperscript{28}

A number of approaches to inhibit oncogene function are being explored in preclinical models. Using antisense oligonucleotides techniques, Jansen et al\textsuperscript{26} blocked the expression of several members of the ras family such as Ha-ras or N-ras in melanoma cells. By injecting Ha-ras-specific phosphorothioate oligonucleotides, these investigators were able to slow the growth of human melanoma cells in severe combined immunodeficient (SCID) mice.\textsuperscript{26} More recently, Putney et al\textsuperscript{29} have targeted c-myc oncogene by administering microencapsulated c-myc-specific antisense oligonucleotides in SCID mice bearing human melanomas. This study showed that a reduction of c-myc expression is associated with reduced tumor growth, decreased number of metastases, and increased survival.
Signal transducers and activators of transcription (STATs) are latent cytoplasmic transcription factors that function as key mediators of cytokine and growth factor signaling pathways. In addition to the central role of STATs in the control of cell proliferation, differentiation, and apoptosis, numerous studies have demonstrated that constitutively activated STAT signaling, particularly Stat3, directly contributes to oncogenesis and malignant progression in human cancers. Indeed, extensive analysis of primary tumors and tumor cell lines indicate that aberrant activation of Stat3 occurs with surprisingly high frequency in a variety of human malignancies, including melanoma. Therefore, Niu et al recently used a gene therapy approach to inhibit activated Stat3 in B16 melanoma cells in vivo. Tumor-bearing mice were electroinjected intratumorally with a vector expressing the dominant negative form of Stat3 (Stat3β) to block endogenous Stat3 signaling in melanoma cells. A significant tumor regression due to massive apoptosis was seen in animals that received dominant-negative Stat3 compared with tumor-bearing mice treated with empty vector. Interestingly, the number of apoptotic cells greatly exceeded the number of transfected cells (10% to 15%), indicative of a potent bystander effect elicited by this gene therapy approach. More recently, these same investigators have shown that the bystander effect could be mediated by soluble apoptotic proteins such as TNF-related apoptosis-inducing ligand (TRAIL), produced as a result of blocking Stat3 signaling in tumor cells (Fig 1).

Similar to the findings observed with other gene therapy approaches, histologic analysis of the tumor site following intratumoral injection of Stat3β revealed an intense infiltration by acute and chronic inflammatory cells. Recently, collaborative studies by investigators at our institute and the Johns Hopkins Oncology Center are providing evidence that cells of the innate as well as adaptive immune system may play a critical role in the in vivo bystander effect associated with gene therapy of certain tumors, specially melanoma. Indeed, inhibition of Stat3 signaling — using either a dominant-negative Stat3β or antisense oligonucleotides — in B16 melanoma cells triggers the production of pro-inflammatory cytokines and chemokines (IFN-β, TNF-α, interleukin IL-6, and IP-10) that activate components of the innate immune system, which ultimately leads to the induction of tumorspecific T-cell responses (H.Yu, et al, unpublished data, 2002). The emerging understanding of the cellular and molecular events involved in the bystander effect of gene therapy is providing the appropriate framework to design strategies to further amplify and sustain this effect. Stat3 is therefore a valid molecular target for developing novel gene therapies against human melanoma, a tumor in which aberrant activation of Stat3 frequently occurs.

**Insertion of Genes Encoding Cytokines or Co-stimulatory Molecules Into Tumor Cells**

Historically, clinical as well as laboratory observations have provided evidence that melanoma is an immunogenic tumor. The higher incidence of this dis-

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**Fig 1. — Inhibition of Stat3 signaling in melanoma cells.**
ease in immunosuppressed patients, the observation of spontaneous regression of primary lesions, and the histologic findings of intense T-cell infiltrate into early melanoma lesions point to cell-mediated immunity as critical in influencing the course of this disease. This finding, together with the identification of melanoma-associated antigens recognized by CD4+ and CD8+ T cells and the demonstration of a small but reproducible clinical benefit (including long-term remissions) in melanoma patients treated with IL-2 or IFNs, has further highlighted the particular immunobiologic properties of this disease.

Techniques allowing efficient gene transfer have permitted the genetic modification of melanoma cells to either secrete immunologically relevant cytokines locally or to express new or increased levels of cell membrane molecules. With this approach, the immunogenicity of melanoma cells is increased by either enhancing the presentation of tumor antigens and/or by providing enhanced co-stimulatory signals to the T-cell arm of the immune system. In preclinical models, these strategies prime systemic immune responses capable of rejecting a subsequent tumor challenge or eradicate established micrometastatic tumors. A systematic comparison of 10 different cytokines or cell surface molecule-based tumor vaccines showed that immunization with tumors transduced with a retroviral vector-expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) produced the greatest degree of systemic immunity, which was enhanced relative to irradiated non-transduced tumors. Priming with GM-CSF-transduced tumor cells led to a potent, long-lived antitumor immunity that required the participation of both CD4+ and CD8+ T cells. Further dissection of the mechanisms mediating this strong antitumor effect showed that GM-CSF produced at the vaccine site promotes the recruitment and activation of the host’s antigen-presenting cells that efficiently uptake, process, and present tumor antigens to antigen-specific T cells leading to strong antitumor responses (Fig 2). Multiple reports have since confirmed the bioactivity of GM-CSF-transduced tumor cells in a number of different tumor model systems, including melanoma. Based on these preclinical data, the Dana Farber Cancer Institute conducted a phase I clinical trial of vaccination with autologous lethally irradiated melanoma cells engineered to produce human GM-CSF. Similar to the findings in experimental models, histologic examination of the vaccination site in all 21 evaluable melanoma patients showed an intense infiltration with T cells, dendritic cells, macrophages, and eosinophils. Pathologic assessment of distant metastases revealed a dense infiltration with T cells and plasma cells after vaccination but not before. Furthermore, an extensive tumor destruction of at least 80%, fibrosis,
and edema were found in 11 of 16 patients examined. Vaccination with GM-CSF transduced melanoma cells also generated antimalanoma tumor-infiltrating lymphocytes (TILs) as well as a humoral immunity. Despite these encouraging findings, these responses were transient and unable to induce clinical regression.

One critical limitation of vaccination with autologous melanoma cells transfected with cytokines genes is that this approach is highly individualized, “custom-made,” expensive, and labor intensive. Therefore, simpler approaches that could maintain the immunologic activity of paracrine cytokine elaboration are currently being developed. One approach, which takes advantage of the fact that the cytokine does not need to be produced by the tumor itself, involves admixing tumor cells with a generic transduced bystander cell. This approach obviates the need for culture or transduction of each patient’s tumor cells, a factor that limited the production of enough vaccine material for human clinical trials. Another approach currently under clinical investigation uses standardized gene-transduced tumor cell lines as vaccines. The rationale behind this strategy is that some tumor rejection antigens not unique but rather are shared. This type of vaccine is often referred to as allogeneic vaccine because the vaccinating cell line expresses major histocompability complex (MHC) alleles that are foreign (allogeneic) to the vaccinated patient. Because it is now well established that tumor antigens are presented by host bone marrow-derived APCs rather than the vaccinating tumor itself, MHC compatibility between the patient and tumor is not required for this type of allogeneic vaccination. A recently reported proof-of-principle trial demonstrated the activity of this type of vaccine in patients with pancreatic cancer. In addition to GM-CSF tumor cell-based vaccines, a large number of human clinical trials testing the efficacy of either autologous or allogeneic melanoma cells transduced with immunologically relevant cytokine genes (ie, IL-2, IL-4, IL-6, IL-7, IL-12, IFN-γ, and IFN-α), or B7 co-stimulatory genes are underway.

In addition to genetic modification of tumor cells in vitro, different groups have efficiently delivered genes directly into tumor nodules in vivo. The introduction of allogeneic MHC class I genes was among the first approaches undertaken for enhancing the immunogenicity of tumors in vivo. In preclinical studies, Plautz et al injected intratumorally an allogeneic MHC class I plasmid DNA admixed with cationic lipids. Using this strategy, tumor growth was significantly delayed and animals were resistant to a subsequent challenge with the wild-type tumor. These investigators translated this approach into a phase I clinical trial to assess the safety of directly injecting DNA encoding the MHC antigen HLA-B7 into tumor nodules. A DNA-lipo-some complex was used to accomplish the in vivo gene transfer. Five HLA-B7-negative patients with metastatic melanoma received microgram amounts of this complex injected in multiple accessible lymph nodes. One of these five patients also received the vaccine by direct injection into a pulmonary metastasis via a pulmonary catheter. Analysis of posttreatment biopsies revealed the presence of plasmid DNA in 1% to 10% of the tumor cells from the injection site. No apparent toxicity was associated with this strategy, and two of two patients evaluated showed generation of antimalanoma cytotoxic T lymphocytes (CTLs). In addition, regression of a treated lesion as well as uninjected melanoma nodules was observed in one patient.

The Arizona Cancer Center recently conducted a similar trial of HLA-B7 gene therapy in patients with metastatic melanoma. Clinical responses, defined as at least 25% reduction in volume of the injected tumor, were seen in seven of 14 patients. The median survival of the 14 patients was 8.1 months. In the majority of patients, the transferred DNA and HLA-B7 protein were found in posttreatment biopsy samples. Interestingly the plasmid DNA could be detected in the injected tumor as long as 8 weeks (timing of last biopsy). An intense infiltration of CD8+ T cells into the tumor site was noted after gene injection, and functional analysis of T cells revealed a significant proliferative alloresponse to HLA-B7 suggestive of a successful xenogenization of the tumor. Hersh and Stoppeck have recently compiled the data available from four phase I/II studies of intratumoral injection of HLA-B7/lipid complex in patients with metastatic melanoma. Of 36 patients treated in these trials, 36% experienced local tumor regression in the injected nodule, and 19% had evidence of regression of distant uninjected melanoma nodules. Although the mechanisms involved in this antitumor effect are not well understood, it is conceivable that the expression of HLA-B7 molecules by the tumor cells elicits a strong allogeneic immune response that enhances (perhaps via increased local cytokine induction) immunity against bystander tumor antigens.

Direct intratumoral or peritumoral injection of vectors expressing IFN-γ has been shown to result in the induction of local as well as systemic antitumor immune responses against melanoma. Fuji et al recently published the results of a phase I study evaluating the safety and activity of IFN-γ retroviral vector injected intratumorally in 17 patients with metastatic melanoma. Patients received either one cycle of treatment with IFN-γ retroviral vector (a cycle of treatment consisted of five daily injections every 2 weeks) or up to six cycles of treatment. In this study, those patients who received multiple cycles of treatment achieved either stable disease or a partial or complete response of the injected
lesion. Anti-MAGE-A1 and tyrosinase antibodies were significantly elevated in the serum of these patients from baseline to week 16 during treatment. Interestingly, the induction of systemic antibody response correlated with clinical response. Although further studies are warranted to determine the efficacy of this strategy, the results of this study as well as those using direct intratumoral injection of HLA-B7 demonstrated the feasibility and safety of in vivo gene transfer approaches.

Genetically Modified Immune Cells

T cells with the capacity to recognize autologous tumor have been isolated from vaccinated animals as well as from patients with cancer, particularly those with melanoma. The antitumor activity of these tumor reactive T cells was further highlighted by adoptive transfer studies that showed melanoma regression in experimental models. In addition, Rosenberg et al published in 1990 the results of the first human gene transfer study in cancer patients evaluating the expression of the neomycin phosphotransferase gene in TILs. These cells were isolated from melanoma nodules, expanded ex vivo, transduced with a retroviral vector, and then reinfused into patients with metastatic melanoma. In addition to the demonstration of an anti-tumor effect in some patients, this study showed the feasibility and safety of this gene transfer approach targeting immune effector cells. Despite the initial optimism generated by this strategy, the ulcerous findings of low response rates, short response duration, and significant toxicities associated with the concurrent use of high doses of IL-2 — as an attempt to prolong T-cell survival — limit the enthusiasm for adoptive transfer of TILs in patients with melanoma.

More recently, investigators at the Fred Hutchinson Cancer Research Center have developed an interesting approach to increase the antitumor efficacy and survival of CD8+ T cells while avoiding the significant side effects associated with IL-2 administration. They used a retroviral vector encoding a chimeric receptor (cytoplasmic domain of IL-2 receptor/extracellular domain of GM-CSF receptor) to render CD8+ T cells helper-independent so that concurrent exogenous IL-2 administration may no longer be required for therapy. This novel approach, together with a better vector technology (i.e., lentiviral vectors) and an increased capacity to isolate and expand T cells ex vivo, is reviving adoptive immunotherapy with genetically modified immune effector cells as a potential strategy in patients with metastatic melanoma. Furthermore, the feasibility to genetically modify stem cells to express T cell receptor genes targeted to specific melanoma-associated antigens provides an additional opportunity for re-directing antigen-specific antitumor immune responses against this disease.

Based on the emerging concept of the central role of APCs in the initiation of immune responses, dendritic cell (DC)-based gene transfer strategies are under active investigation in experimental models as well as in patients with melanoma. DCs are by far the most potent APCs capable of initiating effective T-cell responses. Many features appear to be responsible for the unique antigen-presenting capabilities of DCs. They express 50-fold higher levels of MHC molecules than macrophages, providing therefore more peptide/MHC ligand for T-cell receptor engagement. DCs also express extremely high levels of co-stimulatory and adhesion molecules critical for T-cell activation. The recent development of in vitro culture techniques allowing the generation of large number of DCs made these cells an attractive target for gene therapy strategies. Indeed, different groups have explored the feasibility of ex vivo transduction of DCs using either RNA or replication-defective recombinant viral vectors. Boczkowski et al used RNA derived from tumor cells admixed with cationic lipids as a strategy to introduce genes encoding tumor antigens into DCs. This approach allowed DCs to efficiently display antigenic epitopes and elicit activation of tumor-reactive T cells. Furthermore, preclinical studies have shown that DCs pulsed with RNA are potent antigen-presenting cells in vivo, capable of reducing the number of lung metastases in an experimental melanoma model.

Additional strategies to genetically modify DCs and enhance their therapeutic efficacy include liposomal transfection, gene gun or viral transfer of genes encoding well-defined tumor-associated antigens and co-stimulatory molecules. Schuler and Steinman were among the first to show that murine DCs genetically modified to express β-galactosidase generated strong antitumor responses in either preventive or therapeutic cancer vaccination models. Reeves et al showed the feasibility of transducing human DCs with the melanoma antigen MART-1. These DCs were able to elicit a strong antigen-specific CTL response and to trigger enhanced cytokine production by MART-1-specific TIL. In addition, Ribas et al have shown that in vivo immunization with DCs transduced with a MART-1/Melan-A recombinant adenoviral vector resulted in the induction of a strong anti-melanoma immunity that was superior to vaccine strategies using tumor cells expressing MART-1. More recently, Kikuchi et al undertook a different approach to enhance the therapeutic efficacy of DCs. Based on the emerging critical role of CD40 ligand/CD40 interaction in the initiation of antigen-specific T-cell responses as well as in the prevention of tumor-induced T-cell unresponsiveness, these investigators
genetically modified DCs ex vivo with a recombinant CD40L adenovirus vector. Intratumoral injection of CD40L-modified DCs into B16 melanoma nodules resulted in sustained tumor regression and survival advantage. Interestingly, tumor regression was observed in both DC-injected nodules as well as at distant metastatic nodules. Although these early results using genetically modified DCs are promising, the growing appreciation of different functional subtypes of DCs together with the increasing number of gene transfer techniques necessitates careful comparative studies to determine the best DC-based strategy that would translate into maximal systemic antitumor immunity in vivo.

Conclusions

Significant progress has been made in the field of gene therapy since the early days when foreign genes were successfully introduced into murine cells by using retroviral vectors. However, optimism that gene transfer into human cells will quickly revolutionize cancer treatment was rapidly tempered by the appreciation of the low efficiency of gene transfer as well as by the limited positive results obtained in human clinical trials using a variety of gene therapy approaches. Nonetheless, these pioneer human studies have shown the feasibility and safety of gene therapy of cancer and have unveiled the obstacles to be overcome to make this approach more efficient. For the strategies of introducing suicide genes, replacing defective tumor suppressor genes, or inactivating oncogenes, incremental progress will need to be made, particularly in the identification of better vector technologies that could selectively and efficiently target tumor cells. Meanwhile, of particular interest are the recent advances in the understanding of the cellular and molecular mechanisms involved in the so-called bystander effect elicited by gene therapy strategies. The demonstration of the prominent role of the innate as well as adaptive immune system in mediating the bystander effect offers a unique opportunity to further amplify and sustain this effect that, in turn, may lead us to overcome the low efficiency of the vector technology currently available.

The challenges ahead lie in the translation of the recent advances of gene therapy into reproducible clinical benefit. This will involve careful optimization of the most promising gene therapy strategy and thoughtful selection of the appropriate patient population. In this regard, the advances made in the identification of genetic abnormalities underlying the progression of malignant melanoma, the identification of melanoma associated antigens, and the easy accessibility to tumor lesions have made melanoma the most suitable disease to translate these new developments and perhaps achieve the long-elusive validation of gene therapy as an effective therapeutic tool against cancer.

References


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