Gene Therapy

New Strategies for Malignant Melanoma

Malignant melanoma, the deadliest of all skin cancers, results from transformation of pigment cells, melanocytes. Whereas surgery is curative for local melanoma, conventional therapies fail to affect metastatic disease, mainly because of chemo- and radioresistance of melanoma cells. Indeed, the prognosis for metastatic melanoma is one of the poorest in medicine.

Because of the systemic nature of the disease, strategies to apply gene therapy, and in particular, tumor-targeting approaches, to the treatment of malignant melanoma have been considered. To this end, two new approaches have been pursued and are described here.

The first, immuno-gene therapy, aims to induce or reinforce the patient's immune system to kill cancer cells throughout the body. The second approach looks to engineer tumor-targeted "molecular missiles" bearing suicide genes or replicating viruses. Specifically, these two strategies capitalize on tumor-associated antigens (TAAs), abundantly described for melanoma, or they target molecules of the pigmentation pathways unique to melanocytes.

The ultimate goal of immunotherapy is to overcome the immune evasion by melanoma cells that prevents the patient's native immune system from eradicating cancer lesions. In this regard, immuno-gene therapy attempts to reinforce or initiate the patient's antitumor immune response, based on recent findings in molecular and cellular immunology.

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One example is the transfer of genes encoding immunoregulatory cytokines. Interleukin-2 and the interferons have shown benefits in melanoma patients. The severe side effects observed after systemic administration of these cytokines, however, might be avoided by local expression after gene transfer to the tumor site.

Other strategies require expression of therapeutic proteins in the tumor cell. Tumor cells often inactivate T cells by presenting TAAs via HLA without costimulatory molecules. Expression of costimulatory proteins after gene transfer can render melanoma cells into competent antigen-presenting cells that activate tumor-targeted T cells. Immuno-gene therapies are currently being investigated in clinical trials involving genes for the cytokines IL-2, 4, 6, 7, and 12, IFN-γ, and GM-CSF and the costimulatory molecule B7.

Other immuno-gene therapy approaches are based on the direct genetic modification of immune regulatory or effector cells. In the first clinical gene transfer study, conducted by Steven A. Rosenberg at the NIH in 1990, tumor-infiltrating lymphocytes were expanded ex vivo, genetically labeled, and then injected into melanoma patients, and they showed long-term persistence in patients' blood and tumor. More recently, artificial T-cell receptors, composed of a tumor-recognizing extracellular domain fused to an intracellular signaling domain, have been expressed after gene transfer to T lymphocytes. By bypassing the endogenous MHC-dependent T-cell receptor, this approach avoids immune evasion by melanoma cells that downregulate MHC expression.

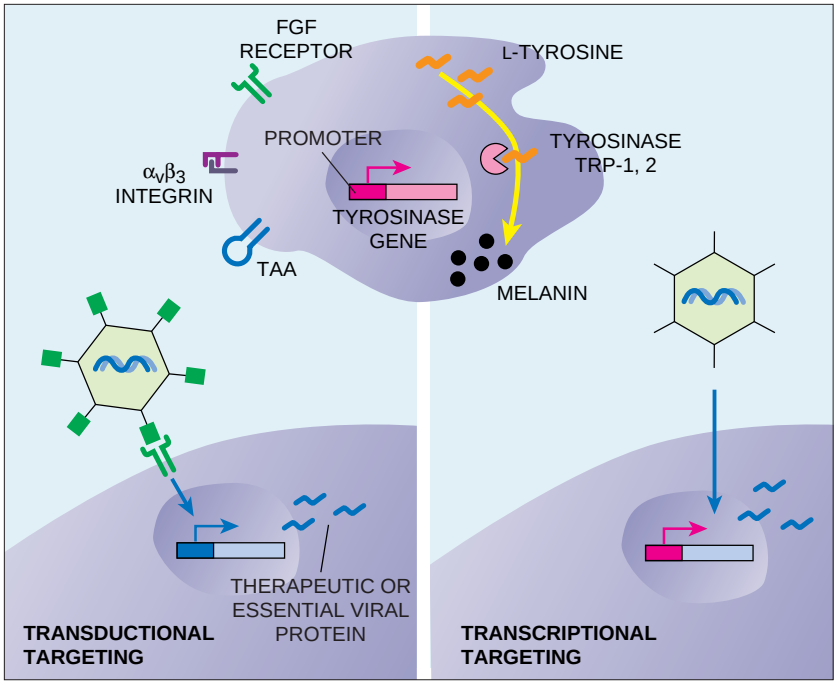
Finally, dendritic cells (DCs), "loaded" with synthetic peptides or tumor lysates, have been used for melanoma vaccination. However, transfer of antigen-encoding DNA or RNA to DCs might offer advantages by allowing endogenous protein expression, modification, and presentation pathways to be utilized, as is often required for successful immune recognition. In the future, direct in vivo gene therapy by means of DC-targeted gene transfer vectors might obviate the need for ex vivo purification and cultivation of patients' cells.

The goal of both molecular chemotherapy and viral oncolysis is direct tumor cell killing. Molecular chemotherapy is based on the transfer of "suicide" genes to tumor cells. These genes encode enzymes that convert a systemically-applied nontoxic prodrug into a locally active, toxic form.

While this strategy aims to mitigate side effects to healthy tissues, the toxicity of the activated drug to neighboring cells (bystander effect) means that gene transfer to each tumor cell is not required. Future research might yield prodrug-activation systems that are tailored to the unique molecular pattern of malignant melanoma.

Viral oncolysis, or virotherapy, is based on tumor cell killing by intracellular virus replication and subsequent cell lysis. Whereas viruses are exploited as gene transfer vectors for gene therapy, viral oncolysis is based on the viral replication cycle and does not require transfer of a therapeutic transgene.

With virotherapy, the therapeutic effect is amplified by the release and spread of progeny virus after tumor cell lysis. The released viruses infect neighboring tumor cells, replicate, and generate the next virus generation, which starts the cycle anew, ideally until all tumor cells are killed.



Targeting of gene therapy to malignant melanoma can be accomplished by exploiting markers unique to melanoma cells. Because melanoma cells express a specific set of surface molecules, such as the FGF receptor, integrins, and TAAs, therapeutic viruses can be targeted to these cells by incorporating into the viral capsid ligands that bind to these surface markers (*left*). Alternatively, it is possible to incorporate specific promoters into the viral vector to mediate the expression of therapeutic or viral genes. These specific promoters are derived from melanoma marker genes, such as surface markers or cytoplasmic enzymes (tyrosinase, TRP-1, and TRP-2) required for pigment synthesis (*right*).

The ability to target these therapeutic "payloads," whether suicide genes or replicating virus genomes, to tumor cells is central to the clinical application of these strategies to melanoma treatment. This challenge has been addressed by efforts to incorporate molecular-targeting devices into viral or nonviral gene transfer vectors or oncolytic viruses.

As mentioned, malignant melanoma has a set of unique differentiation and tumor markers, which have been exploited as targets for molecular cell killing.

One targeting strategy, transductional targeting, mediates binding to specific tumor cells and entry of therapeutic vectors, thus preventing sequestration of these agents by healthy tissues and avoiding toxicities. Targeted gene transfer vectors and tropism-modified viruses have been generated by incorporating tumor-binding ligands as targeting devices.

The most common molecular changes on the surface of melanoma cells are the upregulation of integrins and fibroblast growth factor receptors. Moreover, a panel of surface TAAs and TAA-binding antibodies have been identified for malignant melanoma. For enhanced or targeted infection of melanoma cells, gene therapy has exploited these cell surface markers by using the integrin-binding RGD amino acid motif, growth factors such as fibroblast growth factor, and antibodies to TAAs such as the high-molecular-weight melanoma-associated antigen. Recently, recombinant antibody technologies have been applied.

A second targeting strategy, transcriptional targeting, employs melanoma-specific regulatory DNA sequences (promoters) to drive expression of therapeutic or essential viral genes delivered by gene transfer vectors. In fact, the principle of transcriptional targeting was first shown with the promoter of a prototype differentiation marker, tyrosinase, which is the key enzyme of melanin synthesis and thus exclusive to pigment cells.

This promoter, or its optimized derivatives, mediates strong and highly specific transgene expression and therapeutic effects, with the transgene being a cytokine for genetic immunopotentiation, a suicide gene for molecular chemotherapy, or an essential viral gene for virotherapy.

In addition to promoters of melanocyte-specific genes, the regulatory DNA sequences of genes induced by tumor physiology or conventional treatment regimens are of interest for molecular melanoma therapy. One example is the promoter of the vascular endothelial growth factor (VEGF) gene, which is induced by hypoxia to stimulate blood vessel formation. Hypoxia is a hallmark of solid tumors, and hypoxia-responsive promoters are thus feasible for transcriptional targeting in genetic cancer therapies.

Furthermore, gene therapeutics can be combined with conventional regimens by applying promoters that are induced by irradiation or chemotherapy. This approach is especially promising if the transgene encodes a protein, such as TNF-α, that sensitizes tumor cells to the promoter-inducing therapy.

Clinical trials, now underway, have yet to reveal if the new generation of targeted vectors can translate into successful therapy for melanoma patients.

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