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Gene and immune therapy for renal cell carcinoma

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Abstract Conventional therapy for metastatic renal cell carcinoma is associated with a poor response rate and few patients are long-term survivors. The occurrence of spontaneous regression and the prolonged latency period between primary tumor removal and the appearance of metastases in some patients suggest the existence of important host immune responses to autologous tumor cells. With the advent of molecular gene transfer techniques and increased knowledge of the basic pathways of immune activation, the field of cancer immunotherapy has finally begun to develop novel and effective approaches for harnessing the immune system as a therapeutic agent. Current immunotherapy and gene therapy strategies, including methods of cytokine delivery and tumor-cell-based vaccines, are presented.

Key words cytokine, gene therapy, immunotherapy, renal cell carcinoma, tumor vaccine.

Introduction

For at least 100 years, immunologists have proposed activating the immune system to specifically target and eradicate autologous tumor cells. The idea that tumor cells can be recognized as foreign to the host's immune system is an essential component of tumor immunology. This concept of tumor cell recognition as foreign by their host was first postulated by Paul Ehrlich at the turn of the century. In 1943, Gross noted that when tumor cells were injected subcutaneously into syngeneic mice, the cells formed nodules that grew for a few days and then regressed.[1] When tumor cells were re-injected into the mice, they failed to produce nodules or grow. This was interpreted to mean that the tumor cells did not grow because the mice had become immunologically resistant to the tumor, documenting the existence of tumor-associated antigens. In 1954, Billingham introduced the term 'adoptive immunity' to describe the acquisition of immunity as a result of the transference of immunologically competent cells rather than of preformed antibody.[2] In 1957, Prehn and Main demonstrated, further, that immunization of syngeneic mice with a given tumor protected the mice against a second challenge with the same tumor, but did not protect them from other tumors.[3] In 1959, Thomas suggested that the immune response might be able to rid the body of abnormal cells.[4] His theories were later refined into the 1970 immune surveillance hypothesis of Burnet, which suggests that the immune system could recognize malignant cells as foreign and generate a response against them and that only tumors capable of evading the body's surveillance would be able to grow.[5] In 1972, Borberg successfully documented the ability of adoptively transferred immune cells to cause regression of established syngeneic tumors.[6] Finally, the first successful clinical application of cellular therapy in humans was performed at the National Cancer Institute, Bethesda, USA, by Rosenberg in patients with metastatic melanoma and renal cell carcinoma.[7] Peripheral blood lymphocytes of patients were activated *ex vivo* with IL-2 to generate lymphokine-activated killer cells (LAK) which, when reinfused into the patients, were capable of non-major histocompatibility complex (MHC) restricted tumor lysis.

With the advent of molecular gene transfer techniques and increased knowledge of the regulation of

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being realized. The existence of an immune response against a tumor is based on changes in the surface components of the tumor cell that do not occur in its non-malignant counterpart and that give rise to structures that may be antigenic. Tumor-associated antigens on the surfaces of malignant cells may be unique to the cancerous cells and absent from their normal counterpart, or tumor antigens may be present on normal cells but become unmasked on the malignant cell or represent products that are present during embryonic development but are absent in the normal adult tissue. Immune effector mechanisms capable of destroying tumor cells *in vivo* include humoral (antibody-mediated) and cell-mediated cytotoxicity.

These recent achievements in basic science have not passed over the clinical realm of genitourinary oncology. The use of intravesical immunotherapy for superficial bladder tumors was the first immune-based therapy for bladder cancer and has become the gold standard. Developments in immunotherapy have resulted in an improved outlook for patients presenting with advanced renal cell carcinoma as well as for those who develop both distant and local recurrences after failed curative treatment. These achievements represent only the beginning of new directions to be pursued. The future prospects of cancer therapy will be, without doubt, built upon the foundation of current investigative efforts in gene and immune therapy.

Immune and gene therapy for renal cell carcinoma

Traditionally, there have been no other effective treatments for renal cell carcinoma (RCC) aside from surgery as RCC is radiation- and chemo-resistant. Metastatic RCC has a poor prognosis, with an average survival of only 6–12 months from the time of diagnosis and with only a 6% objective response rate with conventional chemotherapy. Thus, additional therapy, mainly for patients with advanced RCC, is urgently needed. The first advance in this direction occurred when IL-2 was isolated and identified. The molecular cloning of IL-2 revolutionized the field of cancer immunotherapy and significantly altered the treatment of metastatic RCC.[8] With the advent of recombinant DNA technology, the ability to produce large quantities of IL-2 has resulted in its widespread use. In a relatively short period of time, this agent has become a Federal Drug Administration approved treatment for metastatic RCC. Since then, other immunostimulatory cytokines have been identified and purified. To date, focusing on IFN-α, IL-2, combinations of these cytokines, or adoptive immunotherapy with tumor-infiltrating lymphocytes (TIL) or LAK cells. The role of other cytokines (IL-4, IL-7, IL-12 and GM-CSF) is currently under investigation.

Impressive advances have occurred in the past two decades in the application of immunotherapy to treat RCC. At the University of California Los Angeles (UCLA), we have seen a progressive increase in response to treatment as therapy has evolved from systemic IFN-α administration (16%), to combination IFN + IL-2 (25%), to the current method of bulk TIL (33%) and CD8/TIL (40%). Patient characteristics that predict improved responsiveness to therapy have been identified and treatment protocols that decrease toxicity have been developed. The most encouraging results have been the improved rates of complete clinical response, most of which are durable and long-lasting. Further refinements in the treatment of renal cell disease with biologic and immunotherapeutic agents are still needed, yet there is no doubt that current immunotherapeutic protocols produce changes in the natural history of this disease and cause significant and lasting remissions in selected patients. Progress in understanding the genetic changes that are associated with the development of RCC has been made over several years. Thus, gene therapy for RCC has advanced further than in any other urological organ system. Intense efforts and progress have proceeded along several therapeutic paths.

Immunotherapy

It is well established that IL-2 has a beneficial activity in patients with advanced RCC; thus, a tumor vaccine seems appealing. By using a tumor vaccine, elevated cytokine concentrations can be achieved within the tumor causing an increase in MHC expression. By increasing the surface MHC expression, especially of HLA-Cw7, an immune response is anticipated once the TIL are able to recognize the MHC-restricted tumor-related peptides. By achieving locally high cytokine concentrations, the systemic toxicity that limits the efficacy of immunotherapy should be avoided.

Initial studies with tumor vaccines in animal models have shown that the transfer of cytokine genes to tumor cells is feasible and can induce host antitumor effects.[9] IL-2-transfected RCC cells inhibit the growth of parental tumor cells in rats.[9] It has also been shown that the production of IL-2 is more intense after intratumor injection than after systemic administration of

was reported for retroviral transduction of the IL-2 gene conjugated with systemic administration of IFN-α. This suggests that a synergistic, immunogenic effect can be obtained by using both local gene therapy and systemic, immune stimulation. IL-4, GM-CSF, HLA-137 and IFN-α gene transfection are other immune system modulators that may have a role in future tumor vaccines for RCC.[10]

Recent phase I trials using tumor vaccines have been initiated in humans with metastatic RCC. Patients were given irradiated autologous tumor cells transfected *in vitro* with a retroviral vector carrying the GM-CSF gene. No significant toxicity was reported. One out of 16 patients had a partial response. Additionally, studies using genetically modified dendritic cells and studies using the injection of cytokines into the tumor have been performed at UCLA, by using the HLA-B7 and IL-2 genes carried in a liposomal vector. In addition to these, at least three other tumor vaccine programs have been initiated using either intratumoral HLA-B7 or IL-2 gene transfection to enhance the immunogenicity of the tumor. At this time, although tumor vaccine-based gene therapy appears to be safe, its efficacy in metastatic RCC has yet to be proved.

Corrective gene therapy

From studies of familial RCC in patients with the von Hippel–Lindau syndrome, the molecular basis for tumorigenesis of the kidney is becoming clearer; loss of chromosome 3p in many sporadic and familial renal cell cancers has been noted,[11] with restriction fragment length polymorphisms (RFLP). The von Hippel–Lindau (*VHL*) gene was identified at 3p25.5 of chromosome 3.[11] It has been hypothesized that the VHL protein functions as a cell-cycle regulator, controlling cellular proliferation by restricting gene transcription, translation or repair. However, only 45–60% of all patients with sporadic RCC have a detectable mutation in the *VHL* gene. Furthermore, the phenotypic expression of the *VHL* gene defect varies, with loss of the *VHL* gene product not always resulting in RCC.[12] Thus, the defect in the *VHL* gene is probably influenced by many other yet to be defined epigenetic phenomena. Moreover, aberrations at chromosome 5, 7, 14 and at the Y-chromosome have also been associated with RCC. These factors may be able to act independently from the VHL locus, resulting in the development of RCC.

Despite the limitations of the *VHL* gene as a target for gene therapy, initial studies have been performed in product in an attempt to reverse the cancer phenotype. Normal (wild-type) *VHL* gene was transfected into RCC cell lines lacking the normal expression of the gene. The wild-type *VHL* gene was attached to a constitutively activated cytomegalovirus promoter and put into a liposome vehicle. Transfection of the wild-type *VHL* gene had no effects on the transfected cell line growth *in vitro*, but the expression of the wild-type *VHL* gene resulted in growth suppression of other RCC cell lines. This study showed that the suppression of cell growth was specific to RCC cell lines, which implied that the VHL protein is important in controlling the proliferation of kidney cells. Thus, gene replacement therapy using the wild-type *VHL* gene may have a role in treating patients with RCC, although the safety and efficacy of this treatment is yet to be defined.

In vitro attempts to replace the *p53* gene in RCC cell lines using liposome-*p53* gene complexes have resulted in decreased growth of tumor cells in culture. Transfection of the *p53* gene into a mouse-xenograft model resulted in a decrease in the number of metastatic lung lesions.[13] The use of the *p53* wild-type gene by intratumoral injection may prove to be efficacious in the future.

Cyto-reductive therapy

A tumor marker has been recently identified for RCC. This new tumor antigen has been named G250. The function of this protein is unclear. High levels of G250 antigen can be detected in up to 90% of all kidney cancer cells, with normal renal parenchyma showing no detectable G250 antigen. This antigen has been used as a target for monoclonal antibody immunohistochemical staining for diagnostic purposes and has been also used in radionuclide scans to localize tumor sites.[14] Because this antigen is found in a high proportion of RCC cases, it has the potential to be a target for gene therapy. Initial studies looking at cytokine-stimulated human RCC xenografts showed that the antitumor activity of the immune system could be enhanced by the administration of antibodies to G250. For now, it is unknown whether the G250 protein itself can stimulate an immune response. Furthermore, because expression of this gene product is also seen in normal bile ducts and normal gastric mucosa, the safety of an anti-G250 treatment needs to be further tested.

Thus, at this time, there is no perfect target for all patients who have RCC, although restoration of the wild-type *VHL* gene product and the development of targeted therapy against the G250 protein do hold pic-

Conclusion

Although surgical management continues to be an effective treatment for organ-confined neoplastic disease, the treatment options for patients with disseminated cancer are limited. Immunotherapy has demonstrated significant success in the management of advanced RCC. Recent exciting research has demonstrated that prostate cancer may also be susceptible to immuno-therapeutic protocols, finally offering a glimmer of hope to physicians treating this very prevalent and morbid disease. Molecular-based therapy has many potential applications in the treatment of advanced genitourinary cancers. The reinsertion of inactivated tumor suppressor genes, the inactivation of oncogenes, the insertion of immunomodulatory genes and the insertion of suicide genes have all been used to treat genito-urinary malignancies, *in vitro* and in animal models. Progress is being made in better understanding the genetic and cellular mechanisms that underlie tumorigenesis. Human clinical trials are already in phase I testing in some tumor systems, including RCC, transitional cell carcinoma and prostate cancer. However, limitations still have to be overcome. Safe and effective gene vectors will be needed to carry the therapeutic gene to the host cell. Treatments need to be tailored so that the desired effects occur only in the tumor cells. In conclusion, molecular-based therapy is appealing because of its ability to treat cancer at the level of the gene defect that causes the malignant phenotype, and it offers novel and exciting approaches for the treatment and ultimate eradication of cancer.

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