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Brief Communication

Biologic Therapy for Brain Cancers - Based on Cellular and Immunobiology

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The overall goal of our research projects is to develop effective immunotherapeutic regimens, particularly combining vaccine and gene therapy/ cell therapy strategies. For the development of clinically effective immunotherapy for brain cancers, the following issues are considered to be particularly important: 1) Induction of effective immune responses against tumors (afferent arm of the immune response), 2) Delivery of immune effector cells to the target tumor sites and maintaining the activity of the effector cells (efferent arm), 3) For specific and safe immunotherapy, specific brain tumor rejection antigens have to be identified, 4) Feasibility, safety and efficacy need to be tested in a series of clinical trials. The following presentation summarizes my research projects and demonstrates how each plan will fit in the whole schema of designing successful immunotherapeutic strategies for brain cancers.

In this presentation, I would like to focus on our clinical and basic studies related to the vaccine strategies for patients with glioma, and modulation of tumor-microenvironment using bone-marrow derived stroma cells as vehicles for cytokine-gene delivery.

Key Words: Glioma, cancer vaccine, cytokine, bone marrow stroma cells, dendritic cells

Issue 1. Peripheral Vaccine Strategies for Brain Tumors

We have completed a series of pre-clinical studies to demonstrate that peripheral immunization with interleukin (IL)-4 gene inserted glioma cell vaccines induces potent anti-intracranial (IC) glioma activity.[1-4] In addition, we have been able to demonstrate that vaccines composed of tumor-antigen-pulsed dendritic cells (DCs) are effective in controlling the outgrowth of murine IC tumors.[5] These studies have provided pre-clinical bases to develop phase I clinical studies of IL-4 gene transfected glioma cell vaccines to test safety, feasibility and induction of anti-glioma immune responses in patients with malignant gliomas. The first vaccine trial we developed utilize glioma cells transfected with retroviral vector encoding human IL-4.[6,7] Surgically resected glioma tissues are digested as a single cell suspension. This heterogeneous population of cells are cultured and transfected with the retroviral gene vector. Patients receive two cycles of peripheral vaccinations in the thigh. We have enrolled[6] patients to date, completing the scheduled vaccinations in two patients. The first patient demonstrated temporary, but definitive clinical and radiological improvement following vaccinations, with no evidence of allergic encephalitis.[7] Biopsies from the local vaccine sites displayed IL-4 dose-dependent infiltration of immune cells (i.e. $CD4^+$ T cells) at the vaccine sites. As shown in Fig. 1, the most recent subject in this trial has also demonstrated radiological improvement over the course of 6 months following vaccination. The other 3 patients have had to be withdrawn from the trial because of tumor progression, prior to the production of the cellular vaccine. Despite the fact that the primary goal of this phase I study is to prove safety and feasibility of this approach, our interim preliminary data also suggest that immu-

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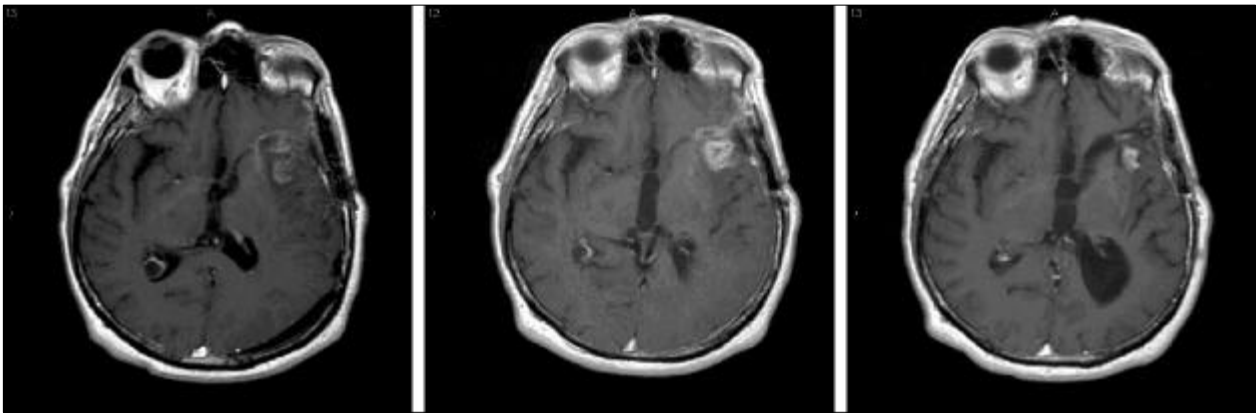

Fig. 1. Regression of recurrent glioblastoma following vaccination with autologous glioma cells admixed with IL-4 transfected fibroblasts. Axial gadolinium-enhanced T1-weighted MR images obtained from the most recently treated patient. The 3-month post-vaccine image (right) shows a remarkable resolution of enhancement in comparison to the pretreatment study (left), which was obtained after surgical debulking but before vaccination, or 1-month post-vaccine (middle). Enhanced lesions were measured independently by a neuro-radiologist.

nologic events are linked with interesting clinical responses, providing a strong rationale for developing novel GAA-based vaccines and the application of modern immunological monitoring strategies to discern potential surrogate endpoints, such as GAA-specific T cell frequencies an function.

It has been suggested that Th1-type $CD4^+$ T cells play critical roles in anti-tumor immunity. It has been revealed that *in vitro* culture conditions for generating monocyte-derived DCs, particularly cytokines, play pivotal roles in polarizing DC in their ability to induce Th1-vs. Th-2 vs. T-regulatory (Tr)-type $CD4^+$ T cell responses to antigenic stimulation.[8-10] We have developed a novel glioma vaccine protocol using DCs that are matured with IFN-γ, TNF-α and IL-1β. These DCs (DC1) are expected to preferentially induce Th1-type T cell responses based on their capacity to produce very high levels of IL-12p70, an essential cytokine for Type-1 responses.[11] This is a pilot study to evaluate the safety, and secondarily, the clinical and immunological efficacy of vaccines (i.e. DC1s loaded with tumor cell lysate and IL-4 expressing fibroblasts) in subjects with malignant gliomas. Local co-expression of IL-4 is expected to promote DC1 maturation and migration of DCs to regional lymph-nodes.[12] The anticipated induction of circulating glioma associated antigen-specific T cells as a result of DC-mediated cross-presentation will be evaluated in the laboratory monitoring aspects of this recently opened trial. We have enrolled one patient to date, with vaccine cells for this patient currently being prepared.

Issue 2. Modulation of the Target Tumor Microenvironment by Delivery of Cytokine Genes in Order to Enhance the Efficacy of Peripheral Vaccines

It has been well established that tumors express immunosuppressive substances that lead infiltrating immune effector cells to apoptotic death and/or incompetent status. Therefore it may be also important to modify the target tumor environment so that local antigen presenting cells and effector cells can maintain their viability and efficacy against tumor cells. Based on our preliminary studies (paper submitted), we believe that certain cytokines (such as interferon-α [IFN-α], among others), produced locally in the brain tumor microenvironment can enhance the activity of therapeutic immunity, particularly in combinational approaches with vaccines.

In order to develop vehicles for delivery of cytokine genes to the infiltrating elements of the brain tumors, we have created a novel cellular vehicle by transfection of murine bone marrow stroma cells (MSCs) with a cDNA encoding epidermal growth factor receptor (EGFR). These cells

(EGFR-MSCs) demonstrate enhanced migratory responses toward glioma conditioned media and recombinant EGF in comparison to primary MSCs *in vitro*. Unlike primary MSCs, EGFR-MSCs were resistant to FasL-mediated cytotoxicity and were capable of stimulating allogeneic mixed lymphocyte reaction, suggesting EGFR-MSCs possess suitable characteristics as vehicles for brain tumor immuno-gene therapy. Following injection at various sites, including the contralateral hemisphere in the brain of syngeneic mice, EGFR-MSCs were able to migrate toward GL261 gliomas or B16 melanoma *in vivo*. Finally, intratumoral injection with EGFR-MSCs adenovirally engineered to secrete IFN-α to intracranial GL261 resulted in long-term survival of host animals at 60%. These data indicate that EGFR-MSCs may serve as attractive vehicles for infiltrating brain malignancies such as malignant gliomas.

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