SURGERY, RADIOTHERAPY AND ADJUVANT DENDRITIC CELL-BASED TUMOR VACCINATION FOR PATIENTS WITH MALIGNANT GLIOMA

THESIS

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Contents

List of Abbreviations

- Ag Antigen
- APCs Antigen presenting cells
- CMI Cell-mediated immunity
- CNS Central nervous system
- CTLs Cytotoxic T lymphocytes
- DCs Dendritic cells
- F Frontal
- FCS Fetal calf serum
- FITC Flourescin isothiocyanate conjugated
- F-P Fronto-Parietal
- GCV Ganciclovir
- GM-CSF Granulocyte macrophage-colony stimulating factor
- HLA Human leukocyte antigen
- IGF Insulin –like growth factor
- IL Interleukin
- INF Interferone
- Lt Left
- MHC Major histocompatibility complex
- MRI Magnetic resonance imaging
- NK Natural killer
- O Occipital
- PBS Phosphate buffer serum
- P-O Parieto-Occipital

- Rt Right
- RT-PCR Reversed transcriptase- polymeraze chain reaction
- RV HSV-tk Retrovirus-mediated herpes simplex virus type 1 thymidine kinase
- T Temporal
- TCRs T cell receptors
- Ths T helper cells
- T-P Temporo-Parietal
- V-P Ventriculo-Peritoneal
- VPC viral producing cells

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Abstract

Malignant brain tumors are among the gravest forms of cancer. Glioblastoma multiforme (GBM), is responsible for 50% of intracranial gliomas and 25% of intracranial tumors in adults. Conventional therapies by surgery followed by radiotherapy and recently with adjuvant chemotherapy still ineffective at improving clinical outcome. Tumor immunotherapy and Cancer vaccines represent one novel therapy for GBM .

Key words : glioma,dendritic cell,immunotherapy,vaccine

Introduction

Malignant brain tumors are among the gravest forms of cancer. The most common of these incurable tumors, glioblastoma multiforme(GBM), is responsible for 50%of intracranial gliomas and 25% of intracranial tumors in adults ***(Davis et al ,2001).*** GBM carries an average survival between 12 and 18 months(with 90-95% patient surviving less than 2 years), without the possibility of spontaneous remission or effective treatment ***(DeAnelis , 2001*)*.*** The consistently short survival and absence of spontaneous remission that makes GBM such a devasting disease also renders the evalution of new therapies for GBM relatively rapid and unequivocal. Overall survival represents the standard by which therapies for GBM are evaluated, in part because tumor mass reduction surgically does not necessarily correlate with prolonged survival ***(Hentschel and Lang ,2003)*** .

In spite of modern oncological treatment, through conventional therapies by surgery followed by radiotherapy and recently with adjuvant chemotherapy still ineffective at improving GBM clinical outcome despite their ability to efficiently treat patients with non glioma tumors ***(Stupp and Hegi ,2003).*** Even the few treatments effective against GBM typically either exhibit small increases in survival that are evident only from large populations studies, or primarily benefit certain patient

subpopulations ***(Dietes et al ,2001).*** Thus , novel therapies that overcome the failings of current GBM treatments are needed.

One of the most recent treatment options is the trial of immune therapy. Brain tumors are considered to be located in a site of relative immune privilege ***(Walker et al, 2002).***Malignant gliomas have immune suppressive characteristics locally and systemically ***(Black et al ,1992)***. In case of vaccination, immune responses are induced at sites remote from tumor. Effector cells recirculate to mediate their antitumor effects in the brain ***(Yu et al ,2001)***.

Cancer vaccines represent one novel therapy for GBM , activated immune cells can survey the entire centeral nervous system with virtually unlimited access since there exists one capillary for every 2 neurons. In addition, activated immune**(T)** cells can cross the blood- brain- barrier **(BBB) T**cell killing of target cells, including tumors, can be exquisitely specific and need not be toxic to normal brain. Moreover,**T** cells retain memory for target (tumor) killing and should reactivate tumor killing if and when recurrence occurs.***(Yu et al, 2001)***.

Tumor immunotherapy, and indeed any immune response against tumors, requires the expression of a target antigen on neoplastic cells. The derivation of tumor antigens was long presumed to be from self molecules altered within neoplastic cells so as to appear “foreign” to the host immune system. It was somewhat surprising ,then that many antigens mediating the rejection of human tumors were found to be essentially

unaltered self molecules involved in routine functioning of the affected tissue ***(Rosnberg,1997)***. This paradox was partially resolved by the realization that tumour cells themselves were not the exlusive in vivo presenters of major histocompatability complex I **(MHC I)-** restricted antigen to immune cells, but this was a function of a specialized group of professional antigen-presenting cells, dendritic cells **(DCs),** that could process self antigen for presentation on **MHC I** ***(Inaba et al ,1990)*** .

Therapeutic vaccination of cancer patients has enjoyed a surge in popularity as an experimental clinical platform with the demonstration that ex vivo- generated **DCs** can stimulate curative anti-tumor **T** cell responses to established tumors in experimental rodents ***(Zitovgel et al ,1996)***.In these model systems, **T** cell responsiveness coincided with treatment efficacy ***(Walker et al ,2000)***.As comparable **DC** populations were identified in humans ***(Romani et al ,1994)***,the notion that similar **DC** vaccines could be used to treat cancer patients gained favor. Early **DC** vaccine clinical trials in lymphoma and melanoma were initiated that provided a backdrop for human tumors such as malignant glioma ***(Yu et al ,2004)***.

Aim of The Work

Cancer vaccines represent one novel form of therapy for malignant glioma. The clinical efficacy of therapeutic vaccination for any human tumor, however, remains controversial. The primary goal of this study is to assess the safety and efficacy of tumor lysate pulsed dendritic cell vaccination to treat patients with glioblastoma multiforme and anaplastic astrocytoma. Adverse events and progression free survival for all patients will be also assessed.

Review of The Literature

Gliomas are the most common form of primary intracranial malignancy. Unfortunately, high-grade gliomas like anaplastic astrocytoma and glioblastoma multiforme (WHO grade III and IV) are the most frequently encountered and carry the worst prognosis ***(Louis et al, 2001).*** The characteristic resistance to treatment shown by high grade gliomas resides in their biological behavior and their location within the central nervous system (CNS). As most cancers, gliomas are subject to constant genotypic and phenotypic alterations that can lead to treatment resistance. Resistant cell populations get selected once a therapy is administered. In addition, most chemotherapeutic agents cannot effectively reach all tumor cells as the blood-brain-barrier limits the penetration of these drugs to brain tumors ***(Lesniak and Brem 2004).*** With respect to surgical treatment, the complete resection of high-grade gliomas remains a virtually impossible task since the nature of these tumors is to infiltrate diffusely within surrounding brain parenchyma ***(Ehtesham et al, 2005).***

Unfortunately, conventional therapies fail to substantially improve GBM clinical outcome despite their ability to confer significant benefits to patients with non glioma tumors ***(Stupp and Hegi, 2003).***

Surgical resection followed by radiation and chemotherapy remains the most effective treatment ***(Lacroix et al, 2001)***, but surgical resection options are limited by the involvement of vital brain structures. Moreover, the clinical impact of surgery followed by radiation on GBM is manifested as a small increase in survival that is evident primarily in large population studies ***(Lacroix et al, 2001)***.

Chemotherapy minimally improves GBM outcome, and does so primarily in young patients ***(Diete et al, 2001)***. These treatment failures stem, in part, from the fact that GBM cells are by nature highly invasive, such that even radical surgical resections leave disseminated, invasive tumor cells, and thus fail to influence recurrence ***(Burger, 1983)***. In addition, the normal brain is indispensable, yet highly sensitive to cytotoxic treatments, limiting effective treatment dosages ***(Ichikawa et al,2000).*** These factors create the need for more specific GBM treatment modalities.

A significant increase in survival of patients with malignant brain tumors is a major goal of therapy and thus, a wide variety of strategies are being explored. Some of the experimental treatments are based on immunotherapy, stem cell therapy, local chemotherapy and radiotherapy ***(Yu et al 2004; Ehtesham et al, 2005)***

In the last few decades, a considerable amount of research dealing with gene therapy for glioma has been conducted *in-vitro* and in animal models. In the case of human studies, the first clinical trials involving gene therapy for gliomas were published in the 1990's. These pioneer studies consisted of retrovirus-mediated herpes simplex virus type 1 thymidine kinase gene therapy (RV HSV-tk) delivered by intratumoral injections of viral producing cells (VPC) followed by systemic administration of ganciclovir (GCV) ***(Kun et al, 1995).*** Since that, number of trials have gathered information regarding the efficacy and safety of this emerging therapeutic approach.

Dendritic Cells:

Dendritic cells (DCs), originally identified by Steinman and his colleagues (1972) represent the pacemakers of the immune response. They are crucial to the presentation of peptides and proteins to T and B lymphocytes and are widely recognized as the key antigen presenting cells (APCs). They are critical for the induction of T cell responses resulting in cell-mediated immunity (CMI) ***(Steinman and cohn,1973).*** The T cell receptors (TCRs) on T lymphocytes recognize fragments of antigens (Ags) bound to molecules of the major histocompatibility complex (MHC) on the surfaces of APCs. The peptide binding proteins are of two types, MHC class I and