



Brain Tumor *focus*

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"OVERCOMING IMMUNE EVASION BY BRAIN TUMORS LINKING INNATE AND ADAPTATIVE IMMUNITY
USING GENE BASED THERAPEUTICS"

Glioblastoma Multiforme (GBM) is the most common primary brain tumor in adults, and has a dismal prognosis. To identify the optimal preclinical model to test gene therapies for GBM, we characterized intracranial human xenografts in nude mice, syngeneic GL26 GBMs (C57BL/6 mice), CNS1 GBMs (Lewis rats) and spontaneous dog GBM, and compared them with human GBM. All GBMs exhibited necroses, neovascularization, pleomorphism, glial markers, tumor infiltration into non-neoplastic brain, and inflammatory cell infiltration. Endothelial proliferation was only observed in dog GBM. I will discuss the efficiency of transgene delivery and therapeutic gene expression using adenovirus serotype 5 (Ad5) in mouse, rat, dog and human glioma cells (cell lines and primary cultures intra-operative biopsies) and determined the expression of Ad receptors (CAR, integrin, MHCI). Although we found high variability in Ad receptor expression levels amongst the different GBM cells, Ad5 mediated therapeutic gene expression was very efficient in all of them. Further, we found no correlation between the levels of CAR, INT or MHCI molecules and levels of transgene expression, or the number of GBM cells transduced. I will also show *in vivo* gene transfer data after administration of Ad5 vectors into the brain of mice, rats and Beagle dogs and widespread distribution of transgene expression in astrocytes and neurons without clinical or neuropathological side effects, attesting to the suitability and efficacy of Ad5 to drive effective and safe therapeutic transgene expression for the treatment of GBM. To identify the most potent cytotoxic gene therapy approach to kill GBM and release tumor antigens *in situ* to be engulfed by immune phagocytic/antigen presenting cells recruited into the tumor mass, I will present data using several Ad5 expressing pro-apoptotic transgenes, i.e. Herpes simplex type 1-thymidine kinase (Ad-TK), TNF-alpha (Ad-TNF-alpha), FasL (Ad-FasL) or TRAIL (Ad-TRAIL). HSV1-TK selectively kills dividing cells in combination with the prodrug ganciclovir (GCV), while TNF-alpha, FasL or TRAIL kill cells expressing the respective death receptor. Ad-TK (+GCV) and Ad-FasL significantly improve the survival of rats bearing established CNS-1 tumors (day 4 after implantation) when compared to saline, Ad-TNF-alpha and Ad-TRAIL. I will also show efficacy data after treatment of larger tumors (day 9 after implantation) with Ad-TK or Ad-FasL alone or combined with Ad-Flt3L (fms-like tyrosine kinase 3 ligand), which recruits and activates dendritic cells into the tumor mass, improving the presentation of tumor antigens released by proapoptotic Ads. The combination of Ad-TK with Ad-Flt3L induced the most significant GBM regression and long term survival. However, since most humans exhibit a pre-existing systemic anti-Ad immune response which could preclude both Ad infection/transduction and eliminate therapeutic transgene expression (Proc Natl Acad Sci U S A. 2000 97: 7482-7487; J Exp Med. 2006 203: 2095-2107), we developed non-immunogenic HC-Ad vectors expressing Flt3L and TK and tested their efficacy in rats bearing large intracranial GBMs. This treatment led to long term survival in 70% of the tumor bearing rats and immunological memory. Our results show that HC-Ad vectors encoding HSV1-TK and Flt3L constitute an attractive therapeutic approach for implementing a clinical trial for human GBM. Finally, I will present new data which demonstrates the role of endogenous TLR ligands released from dying tumor cells in response to TK (+GCV) treatment, which after signaling via TLR2, elicits migration of dendritic cells (DCs) into the tumor mass, antigen uptake, trafficking of DCs (loaded with GBM Ag) to the draining lymph nodes, where they elicit anti-GBM specific T-cells' clonal expansion, tumor regression and long term anti-GBM immunological memory. Our data strongly predict the therapeutic efficacy of our therapeutic strategy in human patients.

PROFILE

Scientific Interests: Dr Castro's research program focuses on understanding the molecular and cellular immune-mediated mechanisms which elicit brain tumor regression with the ultimate goal of developing novel therapeutics and translate them into human clinical trials. The brain is an immune privilege site, which lacks both antigen presenting cells (APCs) and lymphatic drainage, thus hampering effective anti-tumor immune responses. My group is engineering the brain tumor microenvironment utilizing *in vivo* gene transfer technologies to overcome immune privilege in the central nervous system. We are also remodeling the brain microvasculature using tissue engineering technologies to create lymphatic drainage within the brain parenchyma to facilitate migration of immune cells to the draining lymph nodes. We are studying the infiltration of immune cells into the tumor mass and the migration of activated APCs from the brain into the draining lymph nodes. To this end we are using state of the art ex-vivo and *in vivo* imaging technologies, i.e., confocal microscopy, two photon imaging and laser scanning micro-dissection combined with molecular techniques and fluorescently labeled probes. We have uncovered that Toll receptor (TLR) signaling triggered by endogenous tumor derived TLR ligands plays a critical role in the migration of APCs into the tumor mass and in mounting a tumor antigen specific systemic immune response. Our laboratory has recently engineered a single gutless adenovirus based, multi-modality imaging platform, which will enable us to assess disease progression and therapeutic efficacy *in vivo* from mouse rodent cancer models all the way to human patients. This imaging platform encodes molecules which will allow fluorescence, bioluminescence, MRI (without the need of any contrast agents) and PET imaging modalities all under stringent regulation using a genetic switch sensitive to small molecules. My group is currently funded by the NIH and endowments.

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Upcoming Events

Dec 11, 2007
UCLA BTFC

Meeting

Speaker

Frank

Pajonk

2:00-3:30pm

Madden

Conf. Room

13-265 CHS

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