http://neurosurgery.medschool.ucsf.edu/

Collins, continued from page 2

validating. Studies aimed at identifying chimeric transcripts revealed approximately 20 cDNA clones that were predicted to encode chimeric genes; PCR validation of these clones confirmed that three were expressed in the tumor. The experimental validation of these three chimeric transcripts, coupled with computational evidence for many more, suggests that the genomes of brain tumors may encode significant numbers of chimeric transcripts. If confirmed in tissue from additional tumors, these results could have important implications for targeted therapeutics and immunotherapeutic approaches.

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exhibiting subG1 DNA content (a measure of apoptosis) following 24-hour exposure to 0–1000 ng/ml TRAIL. The cells are dissociated, expanded in short-term culture, verified as GBM by comparative genomic hybridization, and assayed by fluorescence-activated cell sorting. These GBM samples are then assayed for expression of FLIP, phosphorylated (activated) Akt, and phosphorylated mTOR by using Western blotting. The concentration of FLIP is compared to TRAIL sensitivity to confirm an inverse relationship.

To determine if inhibition of the Akt-mTOR pathway sensitizes primary GBM to TRAIL-induced apoptosis, aliquots of the short-term cultured GBM cells are incubated with the mTOR inhibitor rapamycin. The cells are then exposed to TRAIL and monitored for levels of phospho-mTOR and TRAIL sensitivity.

The data derived from these studies may prove useful in stratifying patients with GBM for clinical trials of convection-enhanced delivery of TRAIL, as well as helpful for defining methods of circumventing TRAIL resistance using clinically approved mTOR inhibitors.

http://spores.nci.nih.gov/
...A Message From the Director

The University of California, San Francisco’s (UCSF) Neurologic Oncology Program was awarded a Specialized Program of Research Excellence (SPORE) grant in part due to its history of original and significant research into new treatments for brain tumors. The Comprehensive Cancer Center and the Brain Tumor Research Center at UCSF have a long tradition of true bench-to-bedside translational research—research in which scientists and clinicians work in partnership to rapidly translate laboratory findings into new or improved forms of therapy. The format of the SPORE grant was designed to accelerate this process. Projects proposed for a SPORE must be collaboratively designed and executed by physicians experienced in patient-oriented research in tandem with either basic scientists working at the cellular and molecular levels or population scientists experienced in studying the patterns of disease.

One area of research that has proven particularly amenable to rapid translation to the clinical setting has been the development of small-molecule inhibitors. These drugs target aberrant cell-signaling pathways in brain tumors, blocking cell division instead of causing apoptosis. Although traditional cytotoxic drugs can be effective in slowing tumor progression, they can sometimes cause severe side effects because they do not target tumor cells specifically; any dividing cell in the body can be destroyed. Small-molecule inhibitors have a greater specificity for tumor cells, and therefore produce fewer side effects. Investigators David Stokoe PhD and Daphne Haas-Kogan MD, who are leading one of our SPORE projects, have been examining potential small-molecule inhibitors of the phosphoinositide-3-kinase (PI3-kinase) signaling pathway, which is often dysregulated in malignant gliomas. The investigators analyzed the results from a phase I clinical trial of erlotinib (Tarceva) in patients with malignant glioma, and found that patients whose tumors expressed high levels of epidermal growth factor receptor (EGFR) and low levels of phosphorylated PKB/Akt, both components of the PI3-kinase pathway, had the best response to erlotinib. This study was recently published in the Journal of the National Cancer Institute, and will be used to design a phase II trial testing the efficacy of erlotinib in patients with malignant glioma. Selecting treatment for individual patients based on the molecular characteristics of their tumors is an outstanding example of how the SPORE grant is translating molecular research into improved care for patients.

Mitchel S. Berger MD
Director, UCSF Brain Tumor SPORE and UCSF Cancer Center Neurologic Oncology Program

Funded Translational Projects and Investigators

San Francisco Bay Area Adult Glioma Survival Study
Principal Investigator: Margaret Wrensch PhD
Clinical Co-Principal Investigator: Michael Prados MD

Prognostic Value of MRSI Parameters for Patients with Glioma
Principal Investigator: Sarah Nelson PhD
Clinical Co-Principal Investigator: Susan Chang MD

Development of Novel Targeted Therapeutics for Brain Tumor Treatment
Principal Investigator: John Park MD
Clinical Co-Principal Investigator: Mitchel Berger MD

Exploiting the PI3-Kinase Pathway in Human Glioma Therapy
Principal Investigator: David Stokoe PhD
Clinical Co-Principal Investigator: Daphne Haas-Kogan MD

Career Development Awardees

Nalin Gupta MD, PhD
Tumor-associated inflammation as a new target for glioma therapy

Fredric Gorin PhD
The development of novel therapeutic agents directed towards preventing tumor recurrence in malignant gliomas

Developmental Research Awardees

Joseph Costello PhD
New targets for therapy of glioblastoma multiforme unmasked by demethylation

Colin Collins PhD
Identification of chimeric transcripts in brain tumors using end sequence profiling

Russell Pieper PhD
Determining and predicting tumor necrosis factor-related apoptosis-inducing ligand sensitivity in primary glioblastoma multiforme

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Project Title: Identification of Chimeric Transcripts in Brain Tumors Using End-Sequence Profiling

Project Summary: Genomic instability is a hallmark of cancer, and cytogenetic techniques have revealed that brain-tumor genomes carry large numbers of translocations and other complex rearrangements. In an effort to identify chimeric genes that are created from the fusion of two or more normal genes and produce a functional open reading frame, end-sequence profiling (ESP) can be used to perform structural analysis of the genomes and transcriptomes of brain tumors. While established cytogenetic methods such as array comparative genomic hybridization (aCGH) can reveal structural aberrations, their lack of resolution and limited integration with the genome sequence makes identification of specific chimeric genes extremely difficult. To overcome these problems, the Collins laboratory is refining ESP, a technology that allows high-output mapping and simultaneous cloning of all genome rearrangements. ESP has so far been limited to the identification of chromosomal structural rearrangements and has not been able to directly identify the resulting chimeric mRNA transcripts. In this project, ESP will be modified to allow for analysis of transcriptomes, so that chimeric transcripts can be mapped to chimeric genomic clones en masse. Successful modification of ESP will allow for the positive identification of presumed drug targets and associated predictive biomarkers.

Research so far has been performed on tissue from a recurrent tumor (glioblastoma multiforme) for which aCGH and genome-wide methylation data were available. Chromosome copy-number profiles obtained by using aCGH and ESP showed excellent correlation to each other. Numerous likely interchromosomal rearrangements were identified and are now being...
The SPORE grant awarded to UCSF funds four major translational research projects, each driven by a pair of applied and basic researchers, and each intended to create novel tools and therapies potentially useful in the treatment of human brain tumors. In addition, a Career Development Research Program included in the SPORE supports new investigators in the field, and a Developmental Research Program provides initial funding of promising projects that may develop into future SPORE projects.

### San Francisco Bay Area Adult Glioma Survival Study

**Principal Investigator: Margaret Wrensch PhD**  
**Clinical Co-Principal Investigator: Michael Prados MD**

Developing more sophisticated prognostic indicators for glioma and refining tumor classification within and between histological subtypes is essential for developing targeted therapies, and for providing effective therapy to individual patients while sparing them potentially unnecessary treatment. This project is making progress toward these goals by analyzing survival in relationship to both established and novel prognostic factors.

Investigators began by determining the vital status for 873 patients studied in two population-based case series conducted in the San Francisco Bay Area between 1991-1994 (series 1) and between 1997-1999 (series 2) as part of a previous study by Margaret Wrensch PhD. After collecting these data, investigators began analyzing the patients’ survival, evaluating and controlling for variables such as age at diagnosis, ethnicity, tumor histopathological type and grade, and whether or not patients received chemotherapy or radiation therapy. The project is also examining patients’ survival as a function of various potential tumor markers; p53 and p53 mutation analyses, and p53 IHC, MDM2, and EGFR amplification studies on biopsy samples from 500 patients with astrocytomas have been completed.

Other aims of the project have expanded to include promising collaborations with other researchers at UCSF, as well as fellow SPORE recipient University of Alabama, Birmingham (UAB) and the nonprofit organizations Accelerate Brain Cancer Cure (ABC2) and the National Brain Tumor Foundation (NBTF). Investigators from Project 1 have combined their efforts with Project 4 principal investigators Daphne Haas-Kogan MD and David Stokoe PhD to study PTEN methylation in relation to high-grade and low-grade gliomas. They have found that PTEN is methylated in 55% of low-grade tumors, but in only 9% of glioblastoma multiforme (GBM), suggesting that a pathway for PTEN dysregulation other than loss and mutation exists. Based on these findings, PTEN methylation is being examined as a favorable prognostic indicator in GBM.

A second focus of tumor-marker investigation has fostered collaboration with Andrew Parsa MD, PhD and Tarik Tihan MD, PhD at UCSF, who are working to characterize a variety of gliomas with respect to CD8 tumor infiltrates. Wrensch chose 40 patients from the San Francisco Bay Area Adult Glioma Study (SFBAAGS) for CD8 analysis. Initial progress has shown that 35.5% of long-term survivors have increased amounts of CD8 tumor infiltrates, compared with only 12.5% of short-term survivors. These analyses suggest that CD8 tumor infiltrates might be a worthwhile prognostic indicator, and additional patients are being selected for further study in the coming year.

In addition to examining the biology of tumor markers, the project has also made progress in genotyping. Researchers have collaborated with colleagues at UAB to genotype human lymphocyte antigens in blood specimens from patients from the SFBAAGS. Results have shown that GBM is positively associated with HLA genotypes/haplotypes B*13 and B*07-Cw*07 and inversely associated with Cw*01, while HLA A*32 and B*55 were associated with length of survival.

In conjunction with SPORE funding, Wrensch, Prados, and colleagues have also received funding from ABC2 and the NBTF to perform a large-scale genotyping of single-nucleotide polymorphisms in relation to survival and glioma case-control status. They have genotyped 112 patients with glioma and 112 individuals in a control group and have found several promising candidate genes for further exploration.

In the last year, a substantial amount of data has been obtained about the most promising variables that could serve as prognostic indicators for glioma. Investigators have made excellent progress in collecting and categorizing these data, and are now in the process of analyzing them for population science, basic science, and clinical value.