Brain Science Foundation Research Grant Update

Project: Comprehensive identification of therapeutic targets in meningioma

Principal Investigator: Ian F. Dunn, MD

Laboratory: William C. Hahn, MD, PhD, Dana-Farber Cancer Institute

Facilities: Brigham and Women's Hospital; Dana-Farber Cancer Institute; Broad Institute

Background and significance

Despite a few recent successes human cancers, we still lack a comprehensive knowledge of the number and identity of genes whose activity is essential for the transforming events and maintenance of human cancers. This is particularly true in meningioma. Like other solid tumors, meningiomas develop through an accumulation of genetic mutations, facilitated by genetic instability. This instability leads to widespread genetic alterations, which complicates the identification and functional assessment of the critical mutations that drive tumor growth. Thus, to understand the biological basis of malignant transformation and to define cancer targets with the highest promise for clinical translation, we must develop and implement efficient and comprehensive approaches to annotate the cancer genome in functional terms.

Recent advances in genomics make it possible to contemplate enumerating the genetic alterations associated with human cancers. Our laboratory has focused on the design and construction of systematic approaches to discover and characterize the critical mutations that initiate cancer development. We have focused chiefly on such functional genomic approaches as large-scale RNA-interference (RNAi) screening that allow us to "turn off" the majority of genes in the human genome. We can now pair this approach with a global analysis of gene expression, amplifications/deletions, and mutations. Our principal goals in this project are to develop a deeper understanding of the genes that drive meningioma development and growth and to translate these findings into clinically useful therapeutics. Our recent work in glioblastoma, breast cancer, colon cancer, and leukemia has demonstrated that these genomic tools and approaches to the study of cancer genomes may uncover novel targets for therapy[1-8].

Proposal

The lab is part of the RNA-interference consortium (TRC) at the Broad Institute that has created a genome-scale short hairpin RNA (shRNA) library targeting the human genome; with these tools, genes can be "turned off" and their importance to cancer cell growth discerned. Importantly, we have created analytical tools to integrate these RNAi data with information derived from other genomic approaches to enumerate genetic alterations. In our proposal, we planned to derive a comprehensive picture of potential meningioma targets by applying a whole genome, pooled RNAi screening approach to a set of meningioma cancer cell lines. Our aims and the status of each is as follows:

Specific Aim #1: *Identify genes essential for meningioma cancer cell proliferation and survival*
Status: Complete

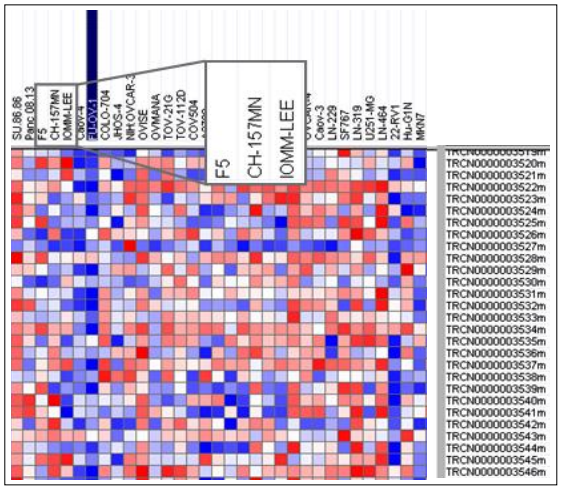
Specific Aim #2: *Identify meningioma oncogenes by integrating functional and structural genomics*
Status: Ongoing

Specific Aim #3: *Functionally validate novel meningioma oncogenes*
Status: To be completed

In Aim 1, we performed whole genome loss-of-function screens in 3 meningioma cell lines (IOMM-Lee, CH-157 MN, F5). These experiments—where over 15,000 genes were "turned off" using RNA-interference—have just recently been completed, and the analysis of the substantial datasets generated is underway. We are ranking these genes by importance to meningioma cell survival using analytical approaches developed at the Broad Institute.

An example of these data are shown in Figure 1, where each column is a different cancer cell line, and each row represents a gene. Three meningioma cell lines are highlighted. The effect of turning each gene off is shown graphically; genes important in the survival of meningioma cell lines are represented by increasing shades of blue. Genes found to diminish meningioma cell survival when turned off are of particular interest.

Figure 1. F5, CH157-MN, and IOMM-Lee meningioma cell growth as a result of "turning off" genes with RNA-interference. In this heat map, each column represents a different cell line, with the meningioma lines highlighted. Each row represents a specific gene turned off. Deeper shades of each blue and red signify cell number as a result of gene targeting (blue=decreased numbers of cells; red=increased numbers of cells).



In Aim 2, we will integrate the information derived from the screens performed in Aim 1 with whole genome analyses of genetic alterations (mutations, copy number and expression) in the same meningioma cell lines. We will identify structural genomic alterations in the same cell lines in which we are conducting shRNA pooled screens using genome-wide single nucleotide polymorphism (SNP) analysis, mutation analysis (using OncoMap[9]), and gene expression profiling. By characterizing structural genetic changes in the cell lines undergoing functional genomic profiling using RNAi, we identify genes that are not only required for proliferation and survival but also are recurrently amplified and/or mutated.

Such genes are likely to represent oncogenes and to be critical drivers of aggressive meningiomas. SNP, OncoMap, and gene expression profiling of meningioma cell lines is ongoing.

In Aim 3, we will functionally validate the best candidate genes identified by these approaches. Specifically, we will determine whether the expression of these candidate genes is necessary for both *in vitro* (anchorage-independent growth) and *in vivo* orthotopic tumor formation. In parallel to these experiments we will determine whether these candidate genes are transforming in both genetically engineered models develop in our laboratory. These studies will not only validate these candidates as potential therapeutic targets but will also provide critical insights into mechanism(s) by which these genes contribute to tumorigenesis.

Relevance to meningioma research

This project will identify and validate meningioma-relevant oncogenes in a comprehensive manner. Oncogenes discovered and authenticated by these studies hold significant promise as therapeutic targets and as such represent a step toward developing more effective cancer treatments for patients with meningiomas. We have completed Aim 1 of our proposal; that is, we have investigated genes essential for the survival of 3 high-grade meningioma cell lines by "turning off" over 15,000 genes using an RNA-interference approach, and our analysis of these results is underway.

With the personnel and technical expertise available to us, we are uniquely positioned to clarify key genes in meningioma. Genes identified in our approach immediately become therapeutic targets. Our long-term goal is to translate an improved understanding of tumor-relevant signaling pathways into the identification of therapeutic targets that will, in turn, ultimately improve the survival of patients diagnosed with these diseases.

Plans for future funding

The project for which I have been funded is to study the function and structure of genes in meningioma cell lines. Currently, our lab is developing integrated methods of using multiple genomic methods in the same cell lines in order to identify cancer-causing genes. In this way, genes identified by more than one genomic method would harbor significant potential as oncogenes. For instance, a gene identified in our RNAi screen as important to cancer cell survival and by our mutation analysis as harboring a mutation would be considered a putative oncogene with potential to serve as a therapeutic target.

Our 3 meningioma cell lines were some of the very first of over 300 cell lines to be analyzed by genome-scale RNAi screening, mutation analysis, gene expression profiling, and global analysis of amplifications and deletions at the Broad Institute. The Brain Science Foundation funding played a critical role in positioning me to accomplish these genomic studies in our group.

Moreover, I anticipate using these data acquired with BSF funding support to apply for a K08 mentored NIH grant.

References

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