

The Glioblastoma Multiforme Brain Tumor Research Grant

David M. Sabatini, MD, PhD

Identification of Molecular Drivers of Brain Tumor Stem Cell Functions Using a Lentiviral RNAi Screen

Whitehead Institute for Biomedical Research (MIT)

Abstract

Brain tumors such as glioblastoma multiforme (GBM), like several other types of cancer, have been shown to contain a subpopulation of cancer stem cells. Like normal neural stem cells, brain tumor stem cells possess extensive proliferative potential, long-term self-renewal, and pluripotency – the ability to differentiate into multiple mature cell types. Cancer stem cells are also highly tumorigenic and believed to possess enhanced drug resistance compared to other cancer cells. Due to these properties, brain tumor stem cells appear to be crucial drivers of GBM initiation and recurrence. To develop curative therapies against GBM, the question that must be answered is what genes expressed in brain tumor stem cells allow them to self-renew, differentiate, and initiate tumor formation. To systematically address this question, we have initiated a collaboration with Dr. William Hahn, a molecular oncology expert at the Dana-Farber Cancer Institute (DFCI), and Drs. Angelo Vescovi, Howard Fine, and Harley Kornblum, three world experts in neuro-oncology. Drs. Vescovi, Fine, and Kornblum have provided us with several cell lines that were isolated from patient brain tumors and enriched for the cancer stem cell subpopulation. To identify genes that control brain tumor “stemness” in these cell lines, we will perform a comprehensive loss-of-function screen using a genome-scale library of shRNA hairpins expressed from lentiviral vectors that we have developed. The unique lentiviral shRNA libraries are able to achieve robust, stable knockdown of thousands of human genes in various cell types and can be used to perform arrayed screens, in which each shRNA is tested in an individual well of a multiwell plate. The main accepted assays for cancer “stemness” are image-based, and we will combine the arrayed shRNA screen with automated fluorescence microscopy and high-throughput image analysis, using software that was also developed here, to ascertain candidate drivers of GBM stem cell functions. Over the one-year grant period, we intend to optimize the screening parameters for the GBM stem cell lines, perform the initial genome-scale loss-of-function screen, and undertake validation of the significant “hits” of the screen, using both in vitro outputs of “stemness” and in vivo tumorigenesis studies. We plan to deliver a focused set of validated molecular drivers of brain tumor stem cell behavior, which can then be further investigated in mechanistic studies and evaluated for potential therapeutic targets.