Disrupting Splicing Disrupts Glioblastoma

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Glioblastoma multiforme (GBM) is a malignant brain tumor that is resistant to existing drug and radiation-based therapies. As a result of this resistance, 90% of patients diagnosed with GBM succumb within two years, highlighting the urgent need for new treatment strategies. In a previous study, Ding, *et al*. (2013), reported a kinase-specific shRNA screen that identified BUB1B/BUBR1 as a requirement for GBM cell proliferation but not for normal cells. In the current study reported in*Genes & Development*, graduate student Christopher G. Hubert (Molecular and Cellular Biology Program), and Drs. Robert K. Bradley (Public Health Sciences and Basic Sciences Divisions), James M. Olson (Clinical Research Division), and Patrick J. Paddison (Human Biology Division), along with a team of collaborators, expand this approach to a genome wide shRNA study, identifying the spliceosome component PHF5A as a novel viability requirement for glioblastoma stem cells (GSCs), which may prove to be a candidate therapeutic target for GBM.

Hubert, *et al*. infected GSC and normal neural stem cells (NSC) with pools of shRNA-expressing viruses and then assessed the cells for differences in shRNA content over time. Most of the tested shRNAs either caused no difference or were underrepresented in the NSCs more than the GSCs; however, the authors identified 27 candidate genes which preferentially inhibited GSCs. Seven of these candidate genes were validated by other criteria and in multiple cell lines; the strongest hit was PHF5A.

Among other functions, PHF5A is a core component of the U2 snRNP spliceosome, a nucleoprotein complex necessary for the removal of introns from pre-mRNA after transcription. Deep RNA sequencing (RNA-seq) experiments identified exon skipping or intron retention in hundreds of genes in PHF5A k/d GSCs but not NSCs. These aberrant splicing phenotypes correlated with a subset of exons encoding characteristic sequences in the 3' splice site, leading to severe RNA processing defects in many cell cycle progression genes. Furthermore, this phenotype was replicated with three different small molecule inhibitors of the U2 snRNP complex of the spliceosome or by knocking down known PHF5A binding partners in the spliceosome. Before PHF5A k/d caused GSC cell death, the authors also noticed dramatic G2/M phase cell cycle arrest. Taken together, these data suggest that PHF5A functions to recognize a specific class of exons with distinctive 3' splice sites which are critical for cell cycle progression in GSC.

To evaluate this altered splicing phenotype in vivo, the authors employed a competition experiment in a xenograft GBM model. PHF5A-shRNA expressing GSCs were unable to proliferate and engraft in vivo and exhibited characteristics of cell cycle arrest two days post implantation. The authors also established GBM tumors expressing a doxycycline-inducible PHF5A-specific shRNA diminished to near undetectable levels after doxycycline administration, while control shRNA transduced tumors grew normally. These findings confirm that PHF5A is necessary for both GBM formation and maintenance. Finally, normal NSCs, astrocytes, or fibroblasts immortalized or transformed with the oncogenes Myc or Ras became acutely sensitive to inhibitors that target the PHF5A-containing component of the spliceosome. This finding suggests that inhibition of PHF5A may be generalizable to a wide range of cancers that rely on these oncogenes.

"PHF5A could be an incredibly powerful target in cancer therapeutics, but it is extremely difficult for traditional small molecule therapeutics. It is nuclear, involves protein:protein interactions and there is no enzymatic "pocket" to target. …[W]e intend to embark on a very challenging project to identify peptide therapeutics that cross the blood brain barrier, enter the nucleus, and disrupt the pertinent interactions," said Dr. Jim Olson.

Hubert CG, Bradley RK, Ding Y, Toledo CM, Herman J, Skutt-Kakaria K, Girard EJ, Davison J, Berndt J, Corrin P, Hardcastle J, Basom R, Delrow JJ, Webb T, Pollard SM, Lee J, Olson JM, Paddison PJ. 2013. Genome-wide RNAi screens in human brain tumor isolates reveal a novel viability requirement for PHF5A. Genes Dev. 2013 May 1;27(9):1032-45.

Also see: Ding Y, Hubert CG, Herman J, Corrin P, Toledo CM, Skutt-Kakaria K, Vazquez J, Basom R, Zhang B, Risler JK, Pollard SM, Nam DH, Delrow JJ, Zhu J, Lee J, DeLuca J, Olson JM, Paddison PJ. 2013. Cancer-Specific requirement for BUB1B/BUBR1 in human brain tumor isolates and genetically transformed cells. Cancer Discov. Feb;3(2):198-211.

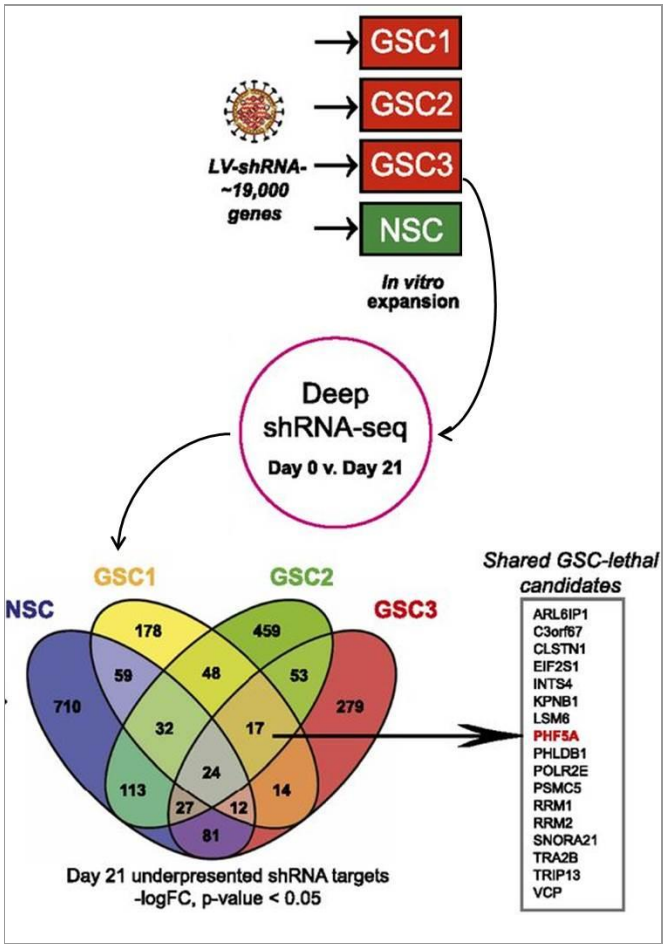

Image modified from Genes & Development

Schematic of the shRNA screen. GSC or NSC cells were infected with a panel of shRNA-expressing viruses. Differential shRNA expression was determined three weeks later to identify genes necessary for GSC but not NSC viability.