

Investigating mechanisms of therapeutic resistance in DIPG

Cynthia Hawkins

The Cure Starts Now, 2013

Brain tumors are the largest group of solid tumors and the leading cause of cancer-related deaths in childhood¹. The most devastating of these is DIPG, an incurable tumor with a median survival of less than one year^{2,3}. DIPGs show poor response to conventional therapy and until recently we have lacked knowledge of the molecular profile of DIPG hindering development of targeted therapeutics. Toward a better understanding of the molecular profile of DIPG, our group at the Hospital for Sick Children was the first to institute a post-mortem tissue collection protocol for DIPG patients and to use this material to obtain high resolution molecular genetic profiles of these tumors. Through high-resolution DNA and RNA microarray analysis of these tumors we uncovered potential therapeutic targets, including *PDGFRA*, *PARP* and *aurora kinase B*^{4,5}, for which therapeutic agents are already available. We have since expanded our protocol to include centers from across Canada and the U.S. and now have one of the largest DIPG tissue banks, with matching clinical data in the world.

Discovery of recurrent histone mutations in pediatric GBM has revolutionized our thinking about the pathogenesis of DIPG⁶⁻⁸. A highly recurrent mutation was found in variant histone H3.3 at amino acid 27 resulting in lysine to methionine substitution (H3.3K27M). While recent data suggests that the presence of the mutated histone results in global loss of methylation of K27 through inhibition of the histone methyltransferase EZH2, how this causes cancer and whether blocking the effects of this mutation can be used to treat DIPG is still unknown. In Aim 1 we propose an siRNA screening approach to identify drugs targeted specifically to DIPGs carrying the K27M mutation. Our siRNA screen is designed to identify genes that are directly targetable through specific small molecule inhibitors.

Despite this huge explosion of genomic data, there has been little movement in identifying which of these mutations, or over-expressed genes in DIPG are bona fide therapeutic targets. Further, we still have little understanding of the reasons for the minimal therapeutic effect of traditional agents, such as radiation and temozolamide, which seem to provide at least some survival benefit for adult patients with high grade astrocytomas. Therefore there is a critical need to understand the basis of this therapeutic resistance in DIPG and translate this into effective treatment protocols for these children.

In adult GBMs, the protein MGMT has been shown to be an important mediator of resistance to temozolamide and patients whose tumors express this protein have a worse overall survival. However, we have demonstrated that MGMT is infrequently expressed in DIPG suggesting an alternate mechanism of therapeutic resistance exists. Another major pathway involved in repairing alkylating-agent mediated damage is the base excision repair pathway. Our data shows that this pathway is highly active in pediatric GBM and that inhibiting this pathway can restore response to temozolamide in both in vitro and in vivo models. We hypothesize that resistance to traditional forms of treatment, namely radiation and alkylating agents such as temozolamide are promoted through the base excision repair pathway in DIPG. The potential for blocking the base excision repair pathway to overcome resistance of DIPGs to radiation and alkylation agents will be tested in Aim 2. The protocols developed in Aim 2 can then be used to test targets from Aim1 in the future.

Our approach will yield highly promising drug targets that can be readily tested in DIPG models and rapidly transitioned to phase I clinical trials. We have the facilities and expertise at the Brain tumor research at The Hospital for Sick Children in Toronto that will allow us to take these preclinical model approaches and eventually transition into clinical trials with our dedicated team of neuro-oncologists. Our innovative approaches of targeting novel DNA repair proteins in combination with standard therapy and our functional siRNA screen will allow us to identify novel therapeutic strategies to treat children with DIPG.