Section 2: Executive Summary

Brain tumors are the largest group of solid tumors and the leading cause of cancer-related deaths in childhood[1]. The most devastating of these is DIPG, an incurable brainstem tumor with a median survival of less than one year[2,3]. DIPGs show poor response to conventional radiation and chemotherapeutic strategies used in adults. Only within the last decade have studies really begun to describe differences between the adult and pediatric disease, underscoring the need for better therapies targeted to the pediatric disease. Most recently, our group reported a major breakthrough in our understanding of DIPG biology with the identification of three molecular subgroups of DIPG[4]: MYCN, Silent and H3K27M. The MYCN subgroup has no recurrent mutations but is instead characterized by DNA hypermethylation, high grade histology, and chromothripsis on chromosome 2p leading to recurrent high level amplification of MYCN and ID2. The Silent subgroup is characterized by silent genomes with a lower mutation rate than the other two subgroups. The H3K27M subgroup is the largest, and all DIPGs in this subgroup harbor a mutation in either *H3F3A* or *HIST1H3B*. In addition, this group is characterized by highly unstable genomes, global DNA hypomethylation and co-occurrence of additional genetic alterations including PDGFRA amplification and *ACVR1*, *PIK3CA* and *TP53* mutations. Discovery of three molecular subgroups has revolutionized our thinking about the pathogenesis of DIPG[6-8] and highlighted the importance of epigenetic dysregulation during tumorigenesis, although it is still unclear which of these alterations represent feasible therapeutic targets. **We hypothesize that combined expression of frequent, subgroup-specific, gene alterations in mouse neural stem cells will result in the dysregulation of normal cellular processes, ultimately leading to malignant transformation.**

In this study we will elucidate the interactions between frequently altered genes in each of the DIPG subgroups and the mechanisms underlying their role in tumorigenesis through two specific aims:

1. Transformation of mouse neural stem cells using lentiviral delivery of genetic alterations associated with each of the three DIPG subgroups.
2. Development of new mouse models of each DIPG subgroup that can be used for pre-clinical drug testing.

Co-expression of subgroup-specific alterations in mouse neural stem cells will allow us to systematically determine the effect of individual alterations on normal cellular processes and their ability to induce malignant transformation and/or maintain the malignant phenotype. Subsequent injection of the mouse neural stem cell lines into the brainstem of immunocompromised mice will enable us to conclude which combination of alterations is both necessary and sufficient for tumor formation and maintenance in vivo. We will then remove the mutant histone to test whether this is important for tumor maintenance in vivo and could thus serve as a therapeutic target.

These experiments will inform production of subgroup-specific transgenic mouse models for DIPG and address a gap in the development of successful DIPG clinical trials. Development of accurate preclinical mouse models of DIPG is crucial to understanding why current therapies fail and for identifying novel drugs that can effectively treat children suffering from this devastating disease. Our group is a leader in the field of DIPG and we are well poised to translate discoveries made through this project into novel and more successful therapeutic trials for this fatal disease.