Mitochondrial DNA alterations and their potential as a novel therapeutic target in DIPG

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Section 2: Executive Summary

Brain tumors are the leading cause of cancer-related death in childhood. Diffuse intrinsic pontine glioma (DIPG) arises in the pons of the brainstem and is universally fatal, comprising nearly 15% of all pediatric brain tumors.[1] Despite international endeavors to improve outcome, DIPGs show poor response to conventional radiation and chemotherapeutic strategies used in adults.[2] Only within the last decade have studies really begun to describe differences between adult and pediatric diseases, underscoring the need for better targeted therapies. Most recently, our group made a major breakthrough in the understanding of DIPG biology by identifying three molecular subgroups: MYCN, Silent and H3K27M.[3] The MYCN subgroup has no recurrent mutations but is characterized by chromothripsis on chromosome 2p leading to amplification of *MYCN* and *ID2*. The Silent subgroup is featured by a lower mutation rate and fewer copy number alterations than the other two subgroups. The H3K27M subgroup is the largest and harbors a K27M mutation in either *H3F3A* (H3.3) or the *HIST1H3* family (H3.1). These findings highlight the potential importance of epigenetic dysregulation in DIPG pathogenesis. However, H3K27M tumors harbor additional alterations while MYCN and Silent subgroup tumors have relatively fewer recurrent genetic alterations, strongly suggesting that H3K27M alone is likely insufficient to drive malignant transformation. Additional mechanisms, including those beyond the traditional epigenetic and genetic modifications within the nuclear genome (nDNA), are likely to be required.

Unlike normal cells that rely primarily on mitochondrial oxidative phosphorylation (OXPHOS) to generate the energy needed for cellular processes, cancer cells depend on aerobic glycolysis to support their uncontrolled growth even in the presence of ample oxygen, a phenomenon termed "the Warburg effect."[4] Abnormal OXPHOS and aerobic metabolism as a result of mitochondrial dysfunction are considered robust metabolic hallmarks of many cancer entities. Numerous somatic mutations in the mitochondrial genome (mtDNA) as well as mtDNA copy number changes have been increasingly observed across a broad spectrum of primary malignancies, including adult brain tumors.[5] Mounting evidence has demonstrated that mtDNA sequence and content variations are associated with neoplastic transformation, tumor progression and metastasis, chemo/radioresistance, and disease prognosis.[6] Due to decreased expression of mtDNA-encoded polypeptides and compromised function of respiratory enzyme complexes, either qualitative or quantitative alterations in mtDNA could elicit a decline in mitochondrial respiratory activity and cause persistent defects in the OXPHOS system accompanied by generation of excessive reactive oxygen species (ROS). This in turn further damages mtDNA, accelerates its mutational rate, and eventually establishes a vicious cycle amplifying mitochondrial dysfunction and oxidative stress. This scenario has been proposed to positively contribute to cancer initiation and/or progression.[5,6]

Despite a huge explosion of DIPG genomic and transcriptomic data and tremendous efforts in characterizing the biological significance of recurrent mutations in nDNA, to date, no study has investigated mtDNA mutations and copy number changes in DIPG or their potential as therapeutic targets. **We hypothesize that mtDNA alterations and consequent impairment of mitochondrial and OXPHOS functions by themselves or in a cooperative fashion with nDNA mutations are important for the initiation and/or progression of DIPG.** Work proposed in this study aims to screen pathogenic "driver" mutations in the entire mitochondrial genome of DIPG tumors by harnessing the advantage of targeted next-generation sequencing (NGS) technology and to elucidate the molecular mechanisms underlying their involvement in DIPG carcinogenesis (**Aim 1**). The potential to exploit altered mtDNA copy number as a novel therapeutic target in DIPG will be evaluated in **Aim 2**. This preclinical work will determine if manipulating mtDNA copy number or stimulating mitochondrial biogenesis is capable of rescuing the metabolic function of defective mitochondria in DIPG and thus reverse the malignant phenotype. Targeted whole mitochondrial genome sequencing may lead to the identification of a set of frequent mtDNA mutations that may play a primary and causative role in DIPG development. Our innovative approach of targeting aberrant mtDNA content in combination with conventional therapies will provide clues for designing novel therapeutic strategies. Our group is a leader in the field of DIPG and being part of the largest pediatric neuro-oncology team in Canada and active contributors in the DIPG collaborative, we are ideally situated to translate discoveries made through this project into novel early diagnostic tools and more effective therapeutic trials for this devastating disease.