Targeting DIPG through pharmacological activation of mitochondrial biogenesis: an *in vitro* and *in vivo* preclinical study

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Section 2: Executive Summary

Diffuse intrinsic pontine gliomas (DIPG) are infiltrative, highly aggressive pediatric brainstem tumors with limited therapeutic options. Despite international efforts to improve outcome, DIPG show poor response to conventional radiation and chemotherapeutic strategies. Only within the last decade have studies really begun to decipher the molecular mechanisms behind DIPG tumorigenesis, with the goal of identifying novel therapeutic targets for this lethal disease. Next generation sequencing studies of DIPGs have identified canonical mutations, most frequently K27M substitutions in either *H3F3A* (H3.3) or *HIST1H3B* (H3.1) as well as *TP53*, *ACVR1*, *PIK3CA* and *PDGFRA*, among others. These results highlight the importance of epigenetic dysregulation in the pathogenesis of DIPG. However, the fact that H3K27M tumors typically harbor additional genetic aberrations and that modeling efforts using histone mutations alone have failed to induce transfomation, strongly supports the notion that H3K27M *per se* is likely insufficient to drive malignant transformation. Additional mechanisms likely exist and are essential for DIPG commencement and/or progression beyond epigenetic and genetic modifications in nDNA.

Unlike normal cells, cancer cells have the capability to make an energy adaption through metabolic reprogramming from oxidative phosphorylation (OXPHOS) to aerobic glycolysis and other metabolic pathways. This occurs regardless of oxygen abundance (the Warburg effect) in order to facilitate their proliferation particularly under unfavorable microenvironments. Abnormal OXPHOS and aerobic metabolism as a result of mitochondrial dysfunction have long been hypothesized to contribute in diverse ways to the multistep process of tumor progression. In recent years, a large number of somatic mutations in the mitochondrial genome (mtDNA) and aberrant mtDNA amount have been increasingly detected in a broad spectrum of primary human cancers. Due to decreased expression of mtDNA-encoded polypeptides and impaired function of respiratory enzyme complexes, quantitative change in mtDNA may decrease mitochondrial respiratory activity and lead to persistent defects in the OXPHOS system. Decreased mtDNA copies and defective mitochondrial function have been strongly linked to neoplastic transformation, tumor progression, metastasis, chemo/radioresistance, and poor prognosis in several types of solid tumors. Our recent work funded by the DIPG Collaborative demonstrated that somatic mtDNA mutations and reduced mtDNA content are highly frequent events in DIPG. Moreover, partial depletion of mtDNA to DIPG-like levels in immortalized NHA (iNHA) cells significantly increased tumorigenicity *in vivo*. These findings led us to ***hypothesize that mitochondrial dysfunction and incompetent oxidative metabolism owing to lower mtDNA copies by themselves, or in a coordinate fashion with nDNA alterations, may be involved in DIPG tumorigenesis*** and DIPG cells may be characterized by "Warburg rearrangement for metabolism".

Work proposed in this study aims to revert the Warburg metabolism and rebuild normal OXPHOS activity in a panel of patient-derived primary DIPG cell lines by targeting reduced mitochondrial number through pharmacological stimulation of PGC-1α-mediated mitochondrial biogenesis (Aim 1). The potential *in vivo* efficacy of three mitochondrial biogenesis drugs (AICAR, resveratrol and metformin) that have excellent profiles and are being used or trialled to treat mitochondrial disorders in other human diseases (e.g. Alzheimer's disease, Type 2 diabetes) will be evaluated in both transgenic murine and human DIPG xenograft models. Through the use of high-throughput synergy drug screening, we will produce preclinical data identifying the best clinically approved compounds to be used in combination with mitochondrial targeting in the chemoadjuvant setting (Aim 2). Our innovative approach of manipulating mtDNA quantity to correct compromised mitochondrial function thereby reversing the malignant phenotype of DIPG, either alone or in combination with conventional therapies, will yield important preclinical data 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 phase I/II clinical trials for this devastating pediatric cancer.