THE HOSPITAL FOR SICK CHILDREN (SICKKIDS)

Cynthia Hawkins
cynthia.hawkins@sickkids.ca
4168135938

555 University Ave
Toronto, Ontario M5G 1X8
Canada

METHIONINE DEPRIVATION AS A THERAPEUTIC STRATEGY FOR DIFFUSE INTRINSIC PONTINE GLIOMA

Translational
DIPG, Childhood (Brain Cancer)
Affiliation: DIPG Collaborative
Requested Funding: $100,000

EXECUTIVE SUMMARY

Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating brain tumor arising in the brainstem of children. Despite current multimodal therapies, DIPG remains incurable with a median survival of less than one year making it is the leading cause of brain tumor-related death in children. Independent efforts by us and

other researchers led to the breakthrough discovery of novel mutations in histone H3 in DIPG (H3K27M), which results in diffuse changes in histone and DNA methylation, and highlighted the importance of epigenetic alterations in this disease. However, while we now understand the genetic underpinnings of DIPG, this has not yet led to a therapeutic breakthrough. In order to better understand the pathways driving DIPG that may be targetable, we undertook transcriptomic and proteomic analyses of DIPG patient samples and compared these with matched normal brains. Among others, we uncovered differences in protein expression of multiple members of the methionine (MET) salvage pathway and metabolism in DIPG. Methionine is an essential amino acid necessary for normal growth and cell function. It contributes to protein synthesis and is the precursor to S-adenosylmethionine (SAM), the principal methyl donor in the cell, that is required for methylation of DNA and histones by a variety of methyltransferases. MET is obtained via dietary intake and is recycled through MET salvage pathways or can be synthesized from homocysteine (HCY).

Previous studies have demonstrated that some cancer cells are unable to maintain high levels of viability in methionine-deficient environments and thus exhibit a MET dependency. As a potential strategy to treat cancer, MET restriction (MR diet) has been explored in animal cancer models as well as in human studies with minimal side effects. Furthermore, an MR diet and depletion of MET with recombinant methionase (rMETase) have been shown to arrest cancer growth and sensitize to chemo and radiation therapy[8]. Clinical trials that included the MR diet have shown promise in the treatment of gastric and colon cancer, melanoma, and glioma[8]. Given the relative feasibility of the approach and its combinatorial effects with other cancer therapies, an MR diet/depletion appears to be a very plausible addition to the DIPG therapeutics strategies.

Given the well-documented deregulation of methylation processes in DIPG, we hypothesize that DIPG cells have a differential MET dependency compared with normal cells, making targeting this pathway an efficient and novel way to treat DIPG. This hypothesis is supported by our preliminary data which shows cell death in DIPG cells in a MET restricted media that cannot be rescued by the addition of HCY. Interestingly, in isogenic cell lines, cells harboring the

H3K27M mutation also die in MET deficient/ HCY supplemented media while wildtype cells are rescued by HCY. We will rigorously test our hypothesis through the following specific aims:

Aim 1. Determine the mechanism of methionine *auxotrophy* in DIPG cells.

The mechanism behind DIPG MET dependency and what effects are on methylation within the cells is unclear. Interestingly, recent investigations of MET dependency in cancer cells raised the possibility that some cancer cells actually have normal levels of MTR and synthesize MET levels comparable to normal cells. However, these cells overuse MET for aberrant trimethylation, leading to hindered growth or death in MET-restricted conditions. Aberrant methylation of DNA and histones is a hallmark of DIPG which may explain why any changes in MET flux may be detrimental to these cells. In addition, an increase in transcription and translation, typical of these cells, may put additional pressure on MET distribution and usage.

In order to understand the mechanism behind MET deficiency in DIPG cells, we propose to study protein synthesis, using mass cytometry (Aim 1.1), and transmethylation rates, using Liquid chromatography-mass spectrometry (LC-MS)- based metabolomics of bioactive molecules (Aim 1.2), in DIPG cells grown under MET restriction compared with normal media. Furthermore, we will assess the effects of MET deficiency specifically on DNA (Epic 850k bead arrays) and histone methylation (LC-MS/MS and ChIPseq) (Aim 1.3) and correlate it with cell fitness.

Aim 2. Establish the effects of a methionine-restricted (MR) diet on DIPG growth *in vivo* as monotherapy and in combination with radiation.

Our preliminary data suggest a high impact of MR on the survival of DIPG cells. Furthermore, the MR diet has been shown to be effective in xenograft models for colon and breast cancer, especially in combination with DNA-alkylating agents and radiation. We hypothesize that the MR diet will be effective in the treatment of DIPG xenografts. Importantly, the blood-brain barrier (BBB) will not present as a problem for this approach. We will test the effect of the MR diet compared with the normal diet on tumor growth and survival for 3 DIPG xenograft models (Aim 2.1). DIPG cells will be stereotactically implanted in the

brainstem of 6-8 week-old athymic nude mice using established procedures. Bioluminescence monitoring of tumor size will be conducted weekly by injecting 100mg D-Luciferin/20g mouse starting a one-week post-injection until completion of the protocol. The mice will be randomized into two groups and fed either a normal diet (control) or MR diet. Mice will be monitored for tumor growth by bioluminescence and sacrificed as per tumor endpoint animal care monitoring guidelines. The effects of the MR diet on tumor growth and overall survival will be measured. Tumors will also be harvested for histologic examination and analysis of methylation status. The effects of the MR diet combined with radiation will also be tested (Aim 2.2). Xenografts will be established as described for Aim 2.1 and mice will be randomized into four treatment groups: normal diet/ mock radiation; normal diet with radiation; MR diet/ mock radiation; and MR diet with radiation.

Impact of this proposal. This proposal aims to elucidate the molecular mechanisms of methionine *auxotrophy* in DIPG cells (Aim1) and test a MET restricted (MR) diet in vivo using xenograft mouse models as a monotherapy and in combination with radiation (Aim2). Through this project, we expect to gain a clearer understanding of the mechanism behind MET dependency in DIPG cells, which will underlie a new protocol establishing the addition of MET restrictions to other therapies in order to achieve a combinatorial effect. Our project is the first to directly address the potential of MR diet alone or in combination with radiation for potential use in the treatment of DIPG patients. In addition, the MR diet is easily translatable by incorporating a vegan diet into a patient's treatment protocols. The facilities and expertise at The Brain Tumor Research Centre and the Hospital for Sick Children as well as our close collaborations within the DIPG Registry Group will help us rapidly advance our findings through pre-clinical investigations and eventually into clinical trials.