Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter know, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Sanjana Kunnikuru April 10, 2024

Disrupting Serine Synthesis for Pediatric Medulloblastoma Therapy

by

Sanjana Kunnikuru

Timothy R. Gershon, MD, PhD.

Advisor

Neuroscience and Behavioral Biology

Timothy R. Gershon,MD, PhD.

Advisor

Patrick W. Cafferty PhD.

Committee Member

Andrea Roeser PhD.

Committee Member

2024

Disrupting Serine Synthesis for Pediatric Medulloblastoma Therapy

by

Sanjana Kunnikuru

Timothy R. Gershon, MD, PhD.

Advisor

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

2024

Abstract

Disrupting Serine Synthesis for Pediatric Medulloblastoma Therapy

By Sanjana Kunnikuru

Medulloblastomas, the most prevalent malignant brain tumor in children, originate from the cerebellum and pose significant challenges due to their propensity to metastasize through the central nervous system. One of the most common types of neurons that resides in the cerebellum, the cerebellar granular neurons, is primarily regulated by the Sonic Hedgehog pathway. Dysregulation of this pathway has been known to contribute to the development of medulloblastomas. The genes, *Phgdh* and *Shmt1*, are expressed during the SHH-driven proliferation, and their patterns of expression suggest that they play an important role in the pathogenesis of tumors. This study sought to 1) understand whether SHH induces PHGDH and SHMT1 and 2) how these genes could affect medulloblastoma growth. Mice were bred to induce a mutant of the Smoothened (SMO) protein, a key player in the SHH pathway, resulting in the development of SHH medulloblastomas, termed G-Smo mice. We utilized Western blotting to quantify PHGDH and SHMT1 protein levels in G-Smo mice injected with vismodegib, an inhibitor of the SHH pathway. To understand how these genes could alter tumor growth, we compared tumors in 1) *G-Smo* mice, 2) *G-Smo/Shmt1-KO*, 3) *G-Smo/Phgdh f/f*, and 4) *G-Smo/P&S-KO*. We sectioned and immunostained brains with tumors using antibodies for the proliferation marker, phosphorylated-Rb, apoptosis marker, cC3, and differentiation marker, NEUROD1. The stained cells were annotated and counted using Tissue Studio. Our study found that SHH induces SHMT1 expression. We also found that there were significantly higher proliferative cells in *G-Smo Phgdh f/f, SHMT1 -/-* compared to *G-Smo* mice and significantly fewer differentiated cells in *G-Smo Phgdh f/f* mice compared to *G-Smo* controls. There were no significant differences in apoptotic cells between genotypes. Further investigation into these mechanisms of tumor metabolism will allow for the development of novel, targeted therapies for medulloblastomas.

Disrupting Serine Synthesis for Pediatric Medulloblastoma Therapy

by

Sanjana Kunnikuru

Timothy R. Gershon, MD, PhD.

Advisor

A thesis submitted to the Faculty of Emory College of Arts and Sciences of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

2024

Acknowledgements

This work was supported by the Gershon Lab at Emory University. I would like to thank my Principal Investigator Dr. Timothy Gershon and my mentors Leon McSwain and Andrey Tikunov for their support of my research endeavors and guidance. I also want to express my gratitude to the UNC Pathology Core for their contributions to my work. Lastly, I would like to thank my Neuroscience and Behavioral Biology Undergraduate Honors Thesis Committee (Dr. Andrea Roeser, Dr. Patrick Cafferty, and Dr. Timothy Gershon) for their expertise and patience throughout this entire process.

Table of Contents

Introduction

Medulloblastoma is the most common malignant brain tumor in children, accounting for almost 20% of pediatric tumors (Mahapatra & Amsbaugh, 2023). Medulloblastomas originate from the cerebellum and due to its proximity to the fourth ventricle which houses cerebrospinal fluid these tumors can easily metastasize to other parts of the CNS. If the tumor obstructs these ventricles and causes cerebrospinal fluid buildup, patients can present with obstructive hydrocephalus leading to increased intracranial pressure (Mahapatra & Amsbaugh, 2023). Other clinical manifestations of medulloblastomas in patients include, ataxia, due to impaired muscle coordination, gait disturbances, and headaches (Mahapatra & Amsbaugh, 2023). The five-year survival rate after initial diagnosis ranges anywhere from 20 to 100 percent, and can be influenced by various factors, such as age, subtype of medulloblastoma, and presence of metastases (Mahapatra & Amsbaugh, 2023).

There are four main types of medulloblastomas: Sonic hedgehog, WNT, Group 3, and Group 4. Each subset of medulloblastomas is modulated by different genes (Mahapatra & Amsbaugh, 2023). Current treatments for medulloblastomas consist of surgical resection followed by radiation and chemotherapy (Mahapatra & Amsbaugh, 2023). However, these current treatments utilizing radiation have drawbacks. Since certain radiation therapies target the entire brain a common adverse side effect is disruptions to hormones in the hypothalamic-pituitary axis, such as decreased thyroid stimulating and adrenocorticotropic hormones (Jackson & Parker, 2023).

Cerebellar Development

The cerebellum contains the most numerous types of neuron in the brain: the cerebellar granule neurons (CGNs). This large population of cells is generated in early

postnatal life through the proliferation of cerebellar granule progenitors (cGNPs); these cells divide repeatedly throughout the first year of life in humans and from postnatal day 1 to 15 (P1 to P15) in mice with a peak in proliferation around P7 (Roussel & Hatten, 2011). During the development of cGNPs, these cells exit the cell cycle in progressively greater numbers and migrate from the external granule cell layer to the internal granule cell layer - along the radial processes of Bergmann glia - where they will begin to differentiate (Roussel & Hatten, 2011). Sonic Hedgehog protein (SHH), secreted by the established Purkinje neurons, induces the proliferation of cGNPs, and excessive mitogenic signaling in GCPs during this critical development period can cause medulloblastomas (Roussel & Hatten, 2011).

Sonic Hedgehog Signaling

SHH signaling is integral to physiologic growth in organ development but has also been implicated in pathologic growth in cancer, due to its potential to induce excessive mitogenic signaling (Carballo et al., 2018). The SHH receptor complex includes two key transmembrane proteins, protein patched homolog 1 *(*PTC1*)* and Smoothened (SMO). PTC1 binds to and inhibits SMO. When the SHH ligand binds to PTC1, it releases SMO, which activates the intracellular signal cascade. Once activated, SMO triggers the production of transcription factors essential for cell proliferation (Carballo et al., 2018).

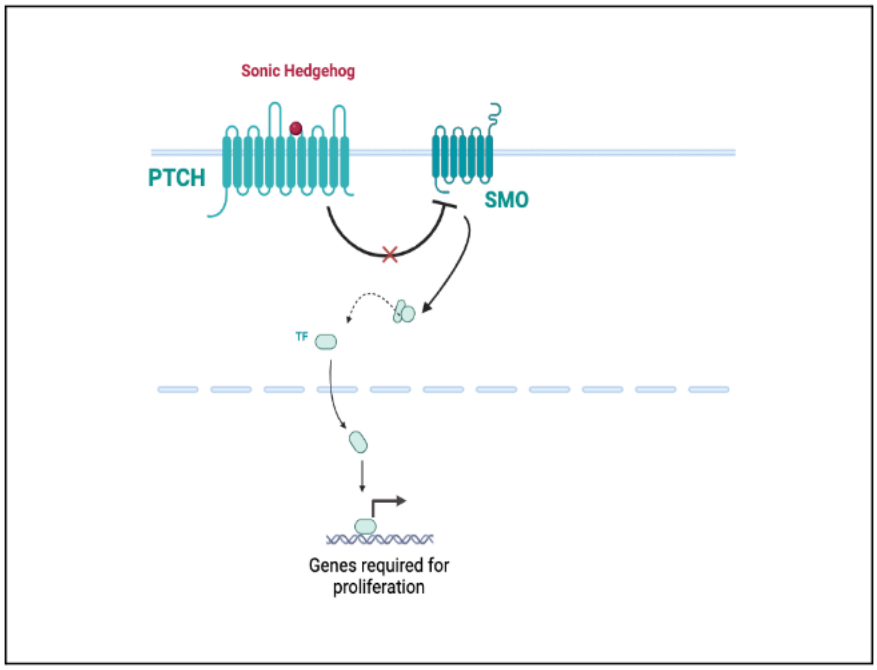

Figure 1a: Sonic Hedgehog Signaling Pathway.

Loss of function mutations that inactivate PTC or gain of function mutation that constitutively active *Smo* can hyperactivate the SHH pathway, ultimately leading to uncontrolled cell proliferation and medulloblastoma formation (Yao et al., 2022, Carballo et al., 2018). Specifically, a mutation at *Smo* 535 Tryptophan (W) into Leucine (L) has been found to induce basal cell carcinomas and medulloblastomas (Yao et al., 2022). *Smo* mutations engineered into transgenic mice that also induce SHH medulloblastomas, providing genetically precise tumor models. For example, mice bred to express both (1)*SmoM2*, a Cre-conditional mutant, constitutively active allele of human Smo, and (2) *Gfap-Cre*, a transgene that induces Cre recombinase in brain stem cells during development where the Cre activates SmoM2 expression in the brain results in SHH hyperactivation and development of SHH medulloblastomas with complete penetrance by postnatal day 10 (Malawsky et al., 2023). These Gfap-Cre/SmoM2 (G-

Smo) mice serve as an efficient model for studying SHH medulloblastoma in a species conducive to experimental investigation, facilitating investigations of the mechanisms of SHH-induced tumorigenesis. Understanding the mechanisms induced by SHH signaling that promote medulloblastoma growth in *G-Smo* mice may lead to new therapies that can target these mechanisms in SHH-driven cancers.

Serine Biosynthetic Pathway

The serine biosynthetic pathway has been found to be important for the synthesis of proteins, nucleic acids, and lipids. Serine provides the building blocks such as methyl groups for the folate pathway. Phosphoglycerate dehydrogenase (*Phgdh*) gene encodes the enzyme responsible for catalyzing the conversion of 3-phosphoglyceric acid, an intermediate of glycolysis, into 3-phosphohydroxypyruvate which contributes to serine synthesis (McKusick, 1998). Subsequent along this molecular pathway is serine/glycine synthesis in which serine hydroxymethyltransferase (*Shmt1*) interconverts serine to glycine, while donating a one-carbon unit from serine to tetrahydrofolate to produce 5, 10-methylenetetrahydrofolate (Sun et al., 2023). Serine/glycine interconversion is a bridge to the folate cycle which supports purine and thymidine synthesis and amino acid homeostasis (Lionaki et al., 2023). Additionally, due to the folate pathway's contributions to these processes, it has been suggested that it plays a key role in cell proliferation and tumor cell viability.

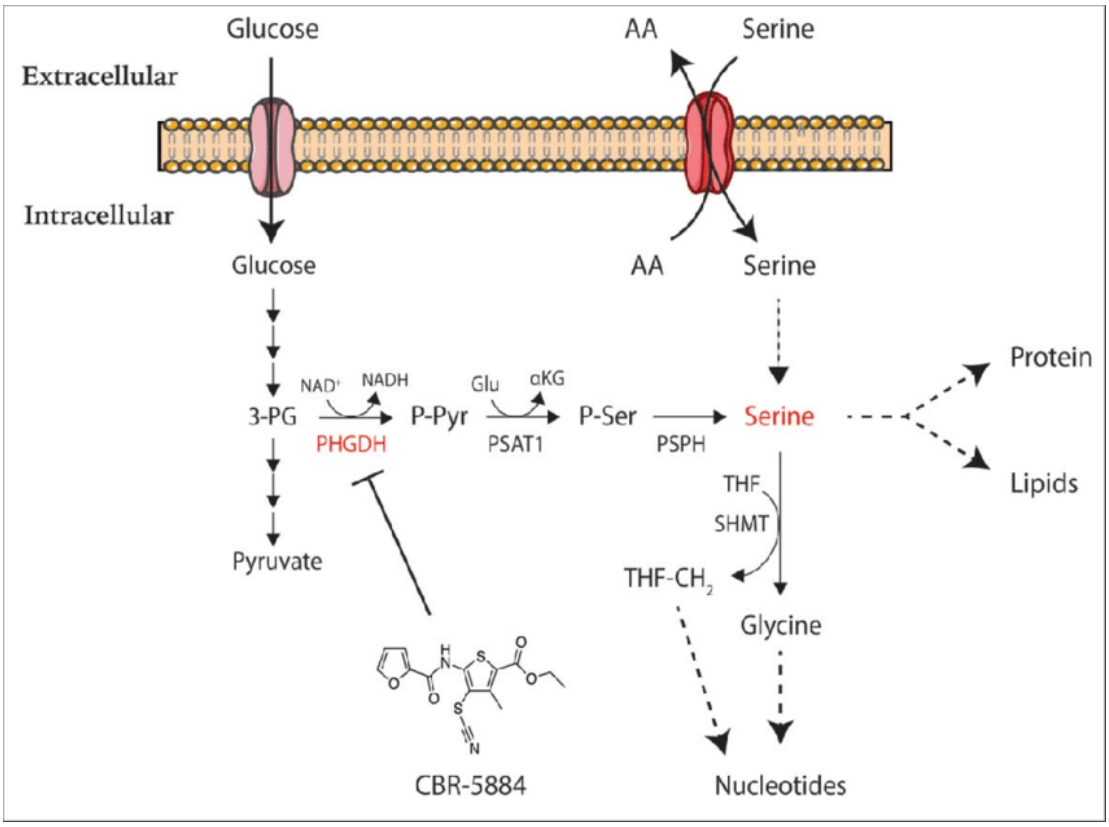

Figure 1b: Serine Synthesis Pathway

Mullarky, Edouard & Lairson, Luke & Cantley, Lewis & Lyssiotis, Costas. (2016). A Novel Small-Molecule Inhibitor of 3-Phosphoglycerate Dehydrogenase. . Molecular & Cellular Oncology. 3. 00-00. 10.1080/23723556.2016.1164280.

The Gershon lab has found that the genes, *Phgdh* and *Shmt1* are expressed by cerebellar progenitors during the period of SHH-driven proliferation and that their pattern of expression suggests these genes may be induced by SHH. *Phgdh* has been implicated in the growth of brain metastases (Ngo et al., 2020), and *Shmt1* has been shown to be important for brain progenitor proliferation (Abarinov et al., 2023). These known functions of *Phgdh* and *Shmt1*, together with their expression in cerebellar progenitors suggest that *Phgdh* and *Shmt1* play an important role in SHH-driven proliferation in normal cerebellar growth and in medulloblastoma.

Current literature on the role of SHMT1 and PHGDH in the development of pediatric medulloblastomas are limited with most research confined to other cancers such as breast cancer. Yet the findings of serine-glycine metabolism in other cancers can be translated to medulloblastomas because increased serine biosynthesis and folate metabolism are observed in most cancers (Mattaini et al., 2016).

In one study that was investigating the impact of *Shmt1* deletion on cognitive decline and neurogenesis compared folate activity levels in mice with a wild-type, heterozygous, and complete deletion of the *Shmt1* gene (Abarinov et al., 2013). They found that there was a 98% reduction in cell proliferation in mice with a *Shmt1* heterozygous genotype compared to wild-type *Shmt1* mice. Additionally, it was found that there was a disruption to normal neurogenesis and differentiation since there was a decreased number of mature neurons in the dentate gyrus (Abarinov et al., 2013). Paone et al., corroborated with the findings of this study and found that *Shmt1* is commonly overexpressed in lung cancer but that there were lower levels of apoptosis during *Shmt1* knockout (Paone et al., 2014).

Previous clinical studies have shown that therapies and drugs targeting folate metabolism may be effective (Zarou et al., 2021). Folate metabolism is essential for the functioning of many processes in the cell including nucleotide synthesis which can help drive cell proliferation. It has been shown that certain enzymes in this pathway are consistently upregulated in cancer, especially SHMT1 (Zarou et al., 2021). Antifolates that actively inhibit folate metabolism have been used to treat leukemia and have demonstrated high sensitivity towards the drug, but unfortunately there are other adverse side effects (Zarou et al., 2021). This highlights the need to better understand

the dynamics behind the folate metabolism in order to create more potent drugs against tumors.

This study seeks to study whether the SHH signaling induces PHGDH and SHMT1 by using a SMO inhibitor to block SHH activation in medulloblastomas that form in G-*Smo* mice, and then studying the effect on PHGDH and SHMT1 proteins. The study will also explore whether *G-Smo* mice bred with genetic alterations of *Phgdh* and *Shmt1*, alter medulloblastoma growth. I hypothesize that vismodegib will decrease the abundance of SHMT1 and PHGDH proteins in cells compared to mice injected with a control DMSO solution. In the second part of the experiment, I hypothesize that deletion of *Phgdh*, *Shmt1*, or both will decrease cell proliferation shown by phosphorylated RB protein. I also hypothesize that these genetic deletions will not produce any significant differences in apoptosis and differentiation demonstrated by cleaved Caspase-3(cC3) and NeuroD1, respectively.

Methods

The subjects used in this study are mice that have been genetically modified to carry a specific mutation in the *Smo* gene, resulting in the development of medulloblastomas in the early stages of their postnatal life. These mice possess two transgenes, *Gfap-Cre* and *SmoM2*. The *Gfap-Cre* transgene prompts the activation of Cre recombinase in brain stem cells during early development, while the *SmoM2* transgene facilitates the Cre-dependent expression of the mutant *Smo* gene. This genetic makeup is referred to as *G-Smo*. The research will focus on four distinct groups of *G-Smo* mice: 1) *G-Smo* mice without any additional modifications (*G-Smo*), 2) *G-Smo* mice with homozygous *Shmt1* null alleles (*G-Smo/Shmt1-KO*), 3) *G-Smo* mice with homozygous Cre-dependent conditional knockout of *Phgdh* (*G-Smo/Phgdh f/f*), and 4) *G-Smo* mice with homozygous null alleles for both *Shmt1* and *Phgdh* (*G-Smo/P&S-KO*). Each group will consist of 3-4 replicate mice for analysis.

Experiment 1 - Does Sonic Hedgehog induce *Phgdh* and *Shmt1*

3 *G-Smo* mice were injected with the SMO inhibitor, vismodegib at P13 and P14 and 3 *G-Smo* mice acted as controls and were injected with DMSO. 3 Control wild-type Black-6 mice (BLK6) were also injected with either vismodegib or DMSO and protein level were compared between these groups as well.

Western Blotting

To ensure that equal lysate concentration was utilized for Western Blotting I used a BSA Assay. I prepared each of the samples by adding 10% beta mercaptoethanol and 90% of Laemmli buffer. The proteins were denatured for 10 minutes at 100 °F and then stored in 80 °C. Proteins underwent separation via gel electrophoresis employing a sodium

dodecyl sulfate polyacrylamide gel followed by a transfer to a polyvinylidene difluoride. I blocked the blots for 30 minutes with 5% Milk BSA solution. I added the primary antibodies for PHGDH and SHMT1(Cell Signaling Technologies, #13428S, #80715) and incubated it overnight at 4°C. I washed the blots with 1X Tris-Buffered Saline with 0.1% Tween 20 Detergent. Secondary antibodies were added and incubated for an hour at room temperature. I visualized the blots with Enhanced Chemiluminescence (ESL) substrate and quantified them with Image J.

Experiment 2 - Does genetic deletion of *Phgdh* and *Shmt1* alter tumor growth

Immunostaining And Quantification

4 mice in each of the following genotype groups, *G-Smo*, *G-Smo/Shmt1-KO*, *G-Smo/Phgdh f/f* and *G-Smo/Phgdh & Shmt1-KO,* were collected and stained using the following primary antibodies: phosphorylated RB(pRB) diluted 1:3000(Cell Signaling #8516), cleaved caspase-3(cC3) diluted 1:50 (Cell Signaling #9664), and Neuronal Differentiation 1(NeuroD1) diluted 1:1000 (Abcam #213725) with the help of the UNC Pathology Core. To conduct immunofluorescence (IF) analysis, the nuclei were stained using 4′6-diamino-2-phenylindole at a concentration of 200 ng/mL for a duration of 20 minutes, followed by the assessment of immunoreactivity utilizing a Leica epifluorescence DM5000B microscope. Each of the stained slides were manually annotated and were cell counted using Tissue Studio (Definiens).

Statistical Analyses

For the NeuroD1 tumor stains, I computed the average cell counts using the sum of (3+) percent nuclei which refers to the degree of staining intensity. The pRb average cell counts for each genotype were computed by averaging the sum of % cell positive. The

cleaved-Caspase 3(cC3) average cell count for each genotype was calculated by summing the 2+ and 3+percent nuclei. I performed a Student's t-test between the genotypes and the *G-Smo* control.

Results

After quantification of the Western Blots using densitometry, I found significantly less SHMT1 protein in the cerebella of vismodegib treated P7 WT mice compared to the DMSO treated control groups ($p<0.05$). I also found significantly lower SHMT1 protein in the medulloblastomas of vismodegib treated *G-Smo* mice compared to the DMSO treated control groups ($p < 0.0001$). I did not find significant differences in the densitometry of the Western blots in the PHGDH proteins between the vismodegib treated and DMSO treated mice in both the wild-type and *G-smo* mice. The finding of decreased SHMT1 in vismodegib treated mice was consistent with my hypothesis that SHH induces Shmt1 expression, while the similar levels of PHGDH between the vismodegib treated and DMSO treated mice was not consistent with my hypothesis.

The analysis of phospho-Rb stained sections of *G-Smo Phgdh f/f*, *G-Smo Shmt1 -/-*, and *G-Smo Phgdh f/f, Shmt1 -/-* mice showed unexpected differences in cell proliferation. I found that the *G-Smo Phgdh f/f, SHMT1 -/-* showed increased percentages of phospho-Rb+ cells compared to G-Smo control mice ($p= 0.008$). These findings of increased phospho-Rb+ cells were contrary to my original hypothesis that PHGDH promotes proliferation.

In the sections of tumors stained for NEUROD1, a marker of terminal differentiation, I found significantly fewer cells with NEUROD1 expression in *G-smo*

Phgdh f/f mice compared to *G-Smo* controls (p=0.035). The decrease in terminally differentiated cells in *Phgdh* deleted tumors was not consistent with my hypothesis that there would not be any differences in NEUROD1 expression between the comparison groups, although it is consistent with the increase in proliferating cells.
Quantification of the cleaved Caspase-3(cC3) tumor stains revealed no statistically significant differences in apoptotic cell counts between the genotypes.

Discussion

Experiment 1

My data show that SHH signaling induces SHMT1 expression in SHH medulloblastomas in mice, where the serine biosynthesis and the serine-glycine one carbon metabolism pathways have consistently been found to have up regulated activity in neuroendocrine prostate, colorectal, breast cancer, and medulloblastomas (Sun et al., 2023). SHMT1 plays a central role in folate methylation, which may be important for medulloblastoma proliferation. Feng et al. found that low folate induced oxidative stress on metabolism caused reduced levels of methylation in the SHH promoter region which increases levels of SHH cascade activation and cell proliferation (Feng et al., 2017) . These prior studies connect folate metabolism to medulloblastoma growth and support the potential role of SHMT1 in medulloblastoma growth.

My data do not support my hypothesis that SHH induces PHGDH expression since there were similar levels of protein in the DMSO and vismodegib treated mice samples. One of the limitations of the study that might explain why a difference was not found is that using western blots on tumor lysates only provides data on the average expression of all cells within the tumor, yet PHGDH is expressed in both tumor cells and

astrocytes (Ehmsen et al., 2013). It is possible that inhibition of Smo using vismodegib only alters PHGDH in only tumor cells or only astrocytes. It is hard to distinguish whether PHGDH expression is similar within tumor lysates of mice treated with vismodegib compared to DMSO unless methods to resolve PHGDH separately between tumor cells and astrocytes are utilized. Additional studies using methods that resolve PHGDH separately in tumor cells and astrocytes will be needed to fully evaluate the effect of vismodegib on PHGDH.

Experiment 2

I found increased phosphoRb+ cells in the *G-Smo Phgdh* f/f, *Shmt1* -/- genotypes compared to the controls. This is in contrast to what I originally hypothesized that *Phgdh* deletion would decrease serine biosynthesis and therefore reduce metabolic products required for cell proliferation. This finding also does not seem to be consistent with the increased survival times of *G-Smo Phgdh f/f* and *Shmt1* -/- knockout mice compared to *G-Smo* mice which would suggest that there are lower levels of cell proliferation which will allow for slower rates of tumor growth and increased longevity. Although previous literature does not corroborate with the findings of the study one possibility that reconciles these findings is that the removal of *Phgdh* and *Shmt1* -/- could initiate compensatory mechanisms where cells utilize exogenous serine more extensively. A more complex hypothesis is needed to unite the findings of increased proliferation and increased survival times in *G-Smo Phgdh* f/f and *Shmt1* -/- tumors.

The expression of NeuroD1 promotes the differentiation of tumor cells (Cheng et al., 2020). Once a tumor cell differentiates, it loses its tumorigenic capacity. Studies that have developed inhibitors to methylation in order to promote NeuroD1 expression have

decreased tumor growth by reducing the number of proliferative cells (Cheng et al., 2020). In this study, I found that the *G-Smo Phgdh f/f* mice had a fewer number of cells expressing NEUROD1 signifying that there are lower numbers of differentiating cells when *Phgdh* is deleted. This corroborates with the results that there are higher proliferative cells because there would be a lower fraction of cells to undergo differentiation.

This study utilized mice with the transgene *Gfap-Cre* to induce the activation of Cre recombination and the expression of the mutant Smo gene. Another mouse model uses the transgene *Math1-Cre* and the mutant Smo gene to generate mice with medulloblastomas (*M-Smo* mice) postnatally. There are specific differences in both these transgenes that could contribute to different clinical outcomes (Wu et al., 2011). *Math1-Cre* specifically targets cerebellar granule neuron progenitors, while *Gfap-Cre* targets neuronal stem cells throughout the brain (Malawsky et al., 2021). Additionally, *M-Smo* mice have a more favorable prognosis with radiation therapy and longer survival times than *G-Smo* mice (Malawsky et al., 2021). These longer survival times of *M-Smo* mice may suggest that there may be differences in tumor metabolism when the *Math1-Cre* transgene is targeted which provide reason to conduct further research that investigates the differences in the impact of SHH inhibition between *Gfap-Cre* and *Math1-Cre* mice on the genes *Phgdh* and *Shmt1* and their protein expression.

To further characterize the neuronal populations within medulloblastomas, as a continuation of this study, we are currently working on staining tumor producing mice with Olig2 and SOX10. Olig2 is a protein expressed by tumor stem cells, while SOX10 is only expressed by oligodendrocytes. Olig2+ identifications in tumor cells are especially relevant because of its role in tumor initiation and recurrence (Malawsky et al., 2021).

Understanding the cellular environment of tumor metabolism enables researchers to discern whether the presence or absence of certain genes yields varied outcomes for tumors.

Conclusion

Serine metabolism has been found to be consistently upregulated in the pathogenesis of tumors, making it a key target for cancer therapeutics (Shunxi et al., 2023). Our findings show that the SHH pathway induces SHMT1, a key enzyme in the conversion of serine to glycine. Genetic deletions of *Phgdh* and *Shmt1* reveal differences in the number of proliferative and differentiating cells in tumors.

Future studies examining the influence of Sonic Hedgehog on serine-glycine and one carbon metabolism could involve creating genotypes that remove folate cycle enzyme intermediates and injecting vismodegib into these mice to observe whether sonic hedgehog plays a larger role in influencing the folate cycle over other enzymes in the serine biosynthesis pathway. Furthermore, studies that involve Edu injected mice can be used to trace cells to determine the distribution of actively dividing neural progenitor cells based on their presence in the G1, S, and G2/M phases in order to further differentiate and examine the role of Phgdh and Shmt1 on cellular metabolism (Flomerfelt & Gress, 2016).

However, there are limitations to this study that can be addressed in follow-up studies. For example, Western Blotting provides protein cell expression throughout all the cells in the brain, but flow cytometry provides more accurate measures of protein expression in different cell populations within the brain. This is pertinent to studying PHGDH because PHGDH is expressed in both astrocytes and neural progenitor cells

and the influence of vismodegib may only impact protein expression in one or both cell types.

There are numerous avenues to further this work and enhance insights into tumor metabolism, especially since it can influence tumor microenvironment and the immune response of tumor cells. Understanding these mechanisms can allow us to find effective, targeted treatments for pediatric medulloblastomas.

References

Abarinov EV, Beaudin AE, Field MS, Perry CA, Allen RH, Stabler SP, Stover PJ. Disruption of shmt1 impairs hippocampal neurogenesis and mnemonic function in mice. J Nutr. 2013 Jul;143(7):1028-35. doi: 10.3945/jn.113.174417. Epub 2013 May 22. PMID: 23700346; PMCID: PMC3681542.

Agostini, M., Antonov, A., Morello, M., Minieri, M., Melino, G., & Amelio, I. (2014). Bioinformatics analysis of the serine and glycine pathway in cancer cells. Oncotarget, 5(22), 11004-11013. https://doi.org/10.18632/oncotarget.2668

Birte Arlt, Guido Mastrobuoni, Jasmin Wuenschel, Kathy Astrahantseff, Angelika Eggert, Stefan Kempa & Hedwig E. Deubzer (2021) Inhibiting PHGDH with NCT-503 reroutes glucose-derived carbons into the TCA cycle, independently of its on-target effect, Journal of Enzyme Inhibition and Medicinal Chemistry, 36:1, 1282-1289, DOI: 10.1080/14756366.2021.1935917

Carballo, G.B., Honorato, J.R., de Lopes, G.P. *et al.* A highlight on Sonic hedgehog pathway. *Cell Commun Signal* 16, 11 (2018). https://doi.org/10.1186/s12964-018-0220-7

Cheng, Y., Liao, S., Xu, G., Hu, J., Guo, D., Du, F., Contreras, A., Cai, K. Q., Peri, S., Wang, Y., Corney, D. C., Noronha, A. M., Chau, L. Q., Zhou, G., Wiest, D. L., Bellacosa, A., Wechsler-Reya, R. J., Zhao, Y., & Yang, Z. (2020). NeuroD1 Dictates Tumor Cell Differentiation in Medulloblastoma. *Cell Reports*, *31*(12), 107782. https://doi.org/10.1016/j.celrep.2020.107782

Choudhry Z, Rikani AA, Choudhry AM, Tariq S, Zakaria F, Asghar MW, Sarfraz MK, Haider K, Shafiq AA, Mobassarah NJ. Sonic hedgehog signalling pathway: a complex network. Ann Neurosci. 2014 Jan;21(1):28-31. doi: 10.5214/ans.0972.7531.210109. PMID: 25206052; PMCID: PMC4117150.

Edouard Mullarky, Luke L. Lairson, Lewis C. Cantley & Costas A. Lyssiotis (2016) A novel small-molecule inhibitor of 3-phosphoglycerate dehydrogenase, Molecular & Cellular Oncology, 3:4, https://doi.org/10.1080%2F23723556.2016.1164280

Ehmsen, J. T., Ma, T. M., Sason, H., Rosenberg, D., Ogo, T., Furuya, S., Snyder, S. H., & Wolosker, H. (2013). d-Serine in glia and neurons derives from 3-phosphoglycerate dehydrogenase. *Journal of Neuroscience*, 33(30), 12464-12469. DOI: 10.1523/JNEUROSCI.4914-12.2013

Feng, C., Lin, Y., Hsu, H., Lan, Y., Kuo, S., Tian, F., Sun, P., & Huang, F. S. (2017). Low folate metabolic stress reprograms DNA methylation-activated sonic hedgehog signaling to mediate cancer stem cell-like signatures and invasive tumour stage-specific malignancy of human colorectal cancers. *International Journal of Cancer*, *141*(12), 2537-2550. https://doi.org/10.1002/ijc.31008

Flomerfelt, F. A., & Gress, R. E. (2016). Analysis of Cell Proliferation and Homeostasis Using EdU Labeling. *Methods in Molecular Biology (Clifton, N.J.)*, *1323*, 211. https://doi.org/10.1007/978-1-4939-2809-5_18

Jackson, K., Packer, R.J. Recent Advances in Pediatric Medulloblastoma. *Curr Neurol Neurosci Rep* **23**, 841–848 (2023). https://doi.org/10.1007/s11910-023-01316-9

Kool M, Jones DT, Jäger N, Northcott PA, Pugh TJ, Hovestadt V, Piro RM, Esparza LA, Markant SL, Remke M, Milde T, Bourdeaut F, Ryzhova M, Sturm D, Pfaff E, Stark S, Hutter S, Seker-Cin H, Johann P, Bender S, Schmidt C, Rausch T, Shih D, Reimand J, Sieber L, Wittmann A, Linke L, Witt H, Weber UD, Zapatka M, König R, Beroukhim R, Bergthold G, van Sluis P, Volckmann R, Koster J, Versteeg R, Schmidt S, Wolf S, Lawerenz C, Bartholomae CC, von Kalle C, Unterberg A, Herold-Mende C, Hofer S, Kulozik AE, von Deimling A, Scheurlen W, Felsberg J, Reifenberger G, Hasselblatt M, Crawford JR, Grant GA, Jabado N, Perry A, Cowdrey C, Croul S, Zadeh G, Korbel JO, Doz F, Delattre O, Bader GD, McCabe MG, Collins VP, Kieran MW, Cho YJ, Pomeroy SL, Witt O, Brors B, Taylor MD, Schüller U, Korshunov A, Eils R, Wechsler-Reya RJ, Lichter P, Pfister SM; ICGC PedBrain Tumor Project. Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. Cancer Cell. 2014 Mar 17;25(3):393-405. doi: 10.1016/j.ccr.2014.02.004. PMID: 24651015; PMCID: PMC4493053.

Li AM, Ye J. The PHGDH enigma: Do cancer cells only need serine or also a redox modulator? Cancer Lett. 2020 Apr 28;476:97-105. doi: 10.1016/j.canlet.2020.01.036. Epub 2020 Feb 4. PMID: 32032680; PMCID: PMC7092752.

Lionaki E, Ploumi C, Tavernarakis N. One-Carbon Metabolism: Pulling the Strings behind Aging and Neurodegeneration. Cells. 2022 Jan 9;11(2):214. doi: 10.3390/cells11020214. PMID: 35053330; PMCID: PMC8773781.

Mahapatra S, Amsbaugh MJ. Medulloblastoma. [Updated 2023 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-.

Malawsky, D. S., Dismuke, T., Liu, H., Castellino, E., Brenman, J., Dasgupta, B., Tikunov, A., & Gershon, T. R. (2023). Chronic AMPK inactivation slows SHH medulloblastoma progression by inhibiting mTORC1 signaling and depleting tumor stem cells. *IScience*, *26*(12). https://doi.org/10.1016/j.isci.2023.108443

Malawsky, D.S., Weir, S.J., Ocasio, J.K. et al. Cryptic developmental events determine medulloblastoma radiosensitivity and cellular heterogeneity without altering transcriptomic profile. Commun Biol 4, 616 (2021). https://doi.org/10.1038/s42003-021-02099-w

Mattaini KR, Sullivan MR, Vander Heiden MG. The importance of serine metabolism in cancer. J Cell Biol. 2016 Aug 1;214(3):249-57. doi: 10.1083/jcb.201604085. Epub 2016 Jul 25. PMID: 27458133; PMCID: PMC4970329.

McKusick, V.A.: Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press, 1998 (12th edition).

Mullarky, Edouard & Lairson, Luke & Cantley, Lewis & Lyssiotis, Costas. (2016). A Novel Small-Molecule Inhibitor of 3-Phosphoglycerate Dehydrogenase.. Molecular & Cellular Oncology. 3. 00-00. 10.1080/23723556.2016.1164280.

Ngo B, Kim E, Osorio-Vasquez V, Doll S, Bustraan S, Liang RJ, Luengo A, Davidson SM, Ali A, Ferraro GB, Fischer GM, Eskandari R, Kang DS, Ni J, Plasger A, Rajasekhar VK, Kastenhuber ER, Bacha S, Sriram RK, Stein BD, Bakhoum SF, Snuderl M, Cotzia P, Healey JH, Mainolfi N, Suri V, Friedman A, Manfredi M, Sabatini DM, Jones DR, Yu M, Zhao JJ, Jain RK, Keshari KR, Davies MA, Vander Heiden MG, Hernando E, Mann M, Cantley LC, Pacold ME. Limited Environmental Serine and Glycine Confer Brain Metastasis Sensitivity to PHGDH Inhibition. Cancer Discov. 2020 Sep;10(9):1352-1373. doi: 10.1158/2159-8290.CD-19-1228. Epub 2020 Jun 22. PMID: 32571778; PMCID: PMC7483776.

Northcott, P. A., Robinson, G. W., Kratz, C. P., Mabbott, D. J., Pomeroy, S. L., Clifford, S. C., Rutkowski, S., Ellison, D. W., Malkin, D., Taylor, M. D., Gajjar, A., & Pfister, S. M. (2019). Medulloblastoma. *Nature Reviews Disease Primers*, *5*(1), 1-20. https://doi.org/10.1038/s41572-019-0063-6

Online Mendelian Inheritance in Man. “Phosphoglycerate Dehydrogenase; PHGDH.” OMIM, https://www.omim.org/entry/606879?search=PHGDH&highlight=phgdh.

Paone, A., Marani, M., Fiascarelli, A. et al. SHMT1 knockdown induces apoptosis in lung cancer cells by causing uracil misincorporation. Cell Death Dis 5, e1525 (2014). https://doi.org/10.1038/cddis.2014.482

Roussel, M. F., & Hatten, M. E. (2011). Cerebellum: Development and Medulloblastoma. *Current Topics in Developmental Biology*, *94*, 235. https://doi.org/10.1016/B978-0-12-380916-2.00008-5

Shunxi, W., Xiaoxue, Y., Guanbin, S., Li, Y., Junyu, J., & Wanqian, L. (2023). Serine Metabolic Reprogramming in Tumorigenesis, Tumor Immunity, and Clinical Treatment. *Advances in Nutrition*, *14*(5), 1050-1066. https://doi.org/10.1016/j.advnut.2023.05.007

Sun, W., Liu, R., Gao, X. et al. Targeting serine-glycine-one-carbon metabolism as a vulnerability in cancers. Biomark Res 11, 48 (2023). https://doi.org/10.1186/s40364-023-00487-4

Twigg SRF, Hufnagel RB, Miller KA, Zhou Y, McGowan SJ, Taylor J, Craft J, Taylor JC, Santoro SL, Huang T, Hopkin RJ, Brady AF, Clayton-Smith J, Clericuzio CL,

Grange DK, Groesser L, Hafner C, Horn D, Temple IK, Dobyns WB, Curry CJ, Jones MC, Wilkie AOM. A Recurrent Mosaic Mutation in SMO, Encoding the Hedgehog Signal Transducer Smoothened, Is the Major Cause of Curry-Jones Syndrome. Am J Hum Genet. 2016 Jun 2;98(6):1256-1265. doi: 10.1016/j.ajhg.2016.04.007. Epub 2016 May 26. PMID: 27236920; PMCID: PMC4908219.

Venneti, S., & Thompson, C. B. (2017). Metabolic reprogramming in brain tumors. Annual Review of Pathology: Mechanisms of Disease, 12(1), 515–545. https://doi.org/10.1146/annurev-pathol-012615-044329

Venneti, S., & Thompson, C.(2016) Annual Review of Pathology: Mechanisms of Disease - Home Page. (n.d.). Annual Reviews. Retrieved from https://www.annualreviews.org/content/journals/10.1146/annurev-pathol-012615-044329

Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron*. 1999;22:103–114.

Wu, X., Northcott, P. A., Croul, S., & Taylor, M. D. (2011). Mouse models of medulloblastoma. *Chinese Journal of Cancer*, *30*(7), 442-449. https://doi.org/10.5732/cjc.011.10040

Zarou, M.M., Vazquez, A. & Vignir Helgason, G. Folate metabolism: a re-emerging therapeutic target in haematological cancers. *Leukemia* 35, 1539–1551 (2021). https://doi.org/10.1038/s41375-021-01189-2

Zhao X, Fu J, Tang W, Yu L, Xu W. Inhibition of Serine Metabolism Promotes Resistance to Cisplatin in Gastric Cancer. Onco Targets Ther. 2020 May 29;13:4833-4842. doi: 10.2147/OTT.S246430. PMID: 32581546; PMCID: PMC7269635.

Figures

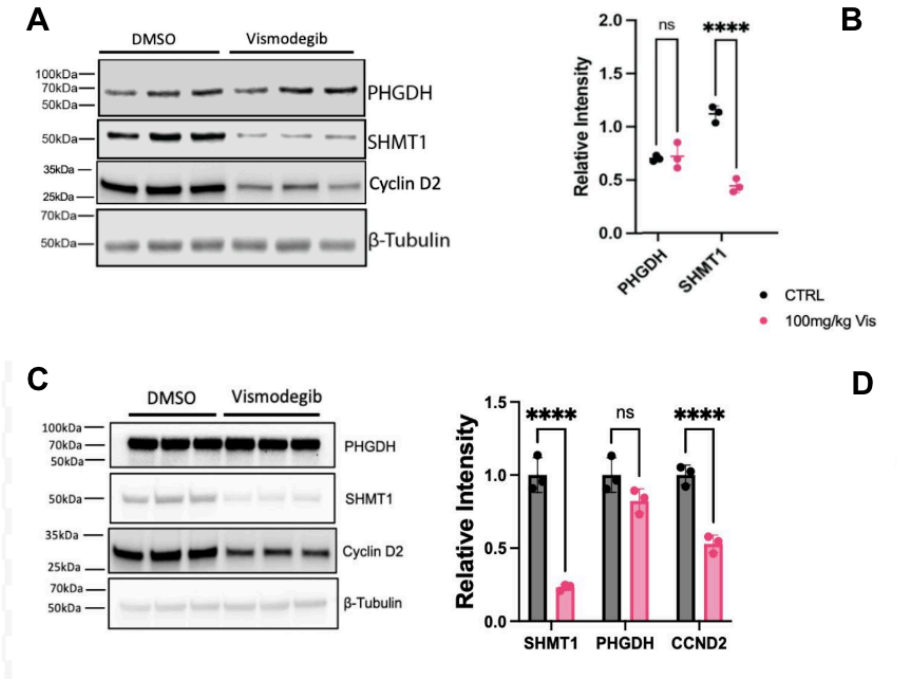

Figure 2 - Comparing PHGDH and SHMT1 protein expression during Sonic Hedgehog Inhibition in wild-type and *G-Smo* mice

(a) Western blots of PHGDH, SHMT1, and Cyclin D2 protein expression in wild-type mice.
(b) Densitometry Quantification of Western Blots in PHGDH and SHMT1 in wild-type mice
(c) Western blots of PHGDH, SHMT1, and Cyclin D2 protein expression in *G-Smo* mice.
(d) Densitometry Quantification of Western Blots in PHGDH and SHMT1 in *G-Smo* mice

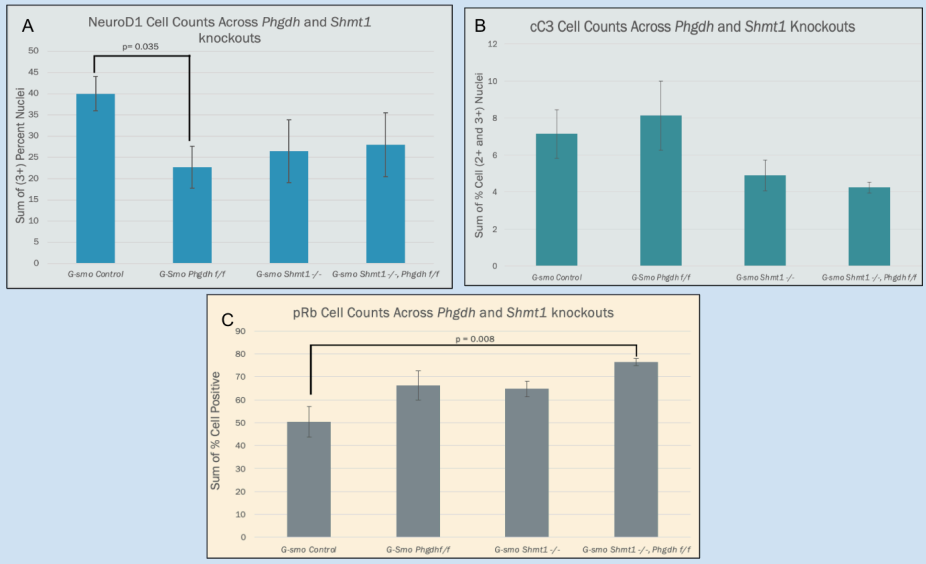

Figure 3: NEUROD1, PhosphoRb, and Cleaved-Caspase 3 Cell Counts in *G-Smo* mice (Error bars using Standard Error of Mean(SEM)

(a)- NeuroD1 differentiation marker stain counts in *G-smo* Control, *G-Smo Phgdh* f/f, *G-Smo Shmt1* -/-, and *G-Smo Shmt1 -/-, Phgdh* f/f mice

(b)- Cleaved-Caspase 3+ cells counts in *G-smo* Control, *G-Smo Phgdh* f/f, *G-Smo Shmt1* -/-, and *G-Smo Shmt1 -/-, Phgdh* f/f mice

(c) - Phospho-Rb+ cells counts in *G-smo* Control, *G-Smo Phgdh* f/f, *G-Smo Shmt1* -/-, and *G-Smo Shmt1 -/-, Phgdh* f/f mice

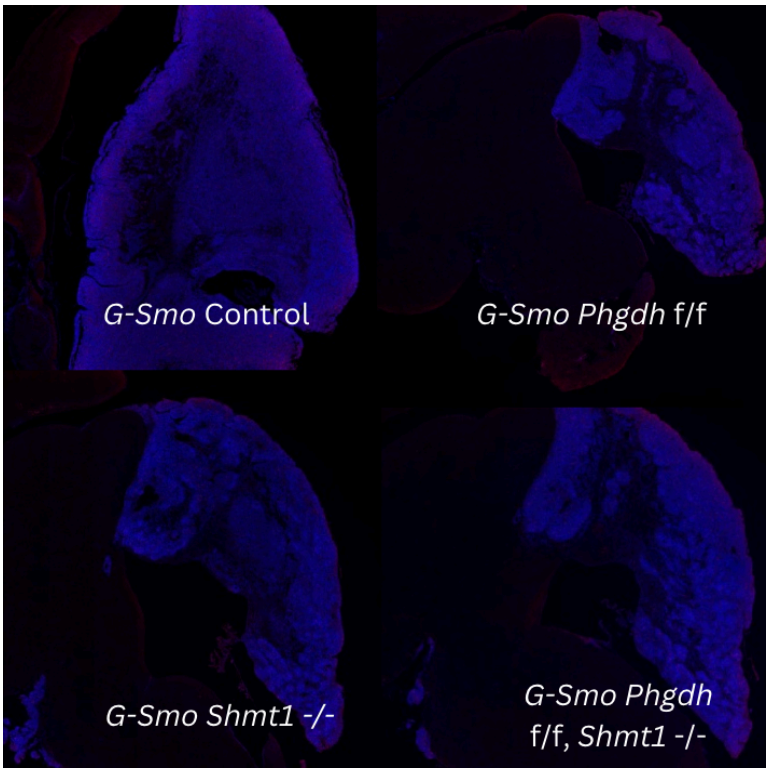

Figure 4: Apoptosis staining in *Phgdh* and *Shmt1* deleted mice
Cleaved Caspase-3 IHC in tumors of *G-smo* Control, *G-Smo Phgdh* f/f, *G-Smo Shmt1* -/-, and *G-Smo Shmt1 -/-, Phgdh* f/f mice

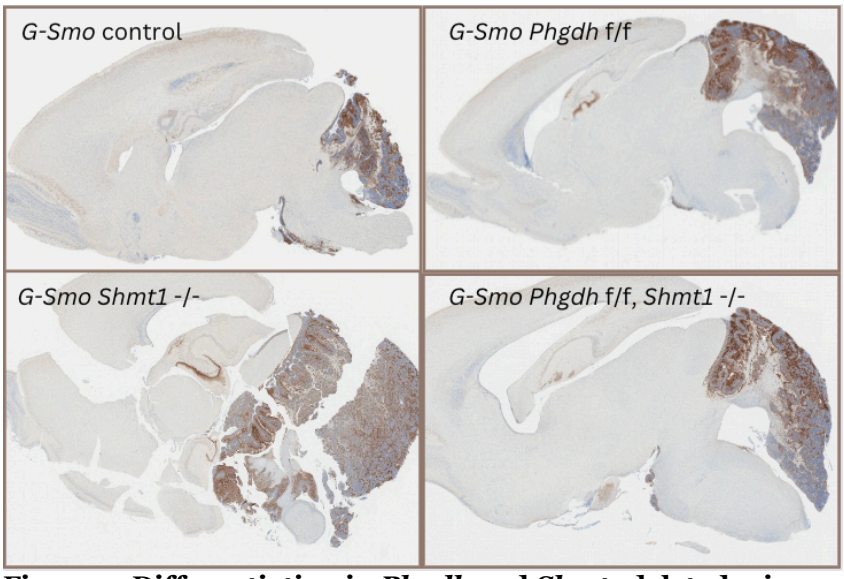

Figure 5: Differentiation in *Phgdh* and *Shmt1* deleted mice
NeuroD1 IHC in tumors of *G-smo* Control, *G-Smo Phgdh* f/f, *G-Smo Shmt1* -/-, and *G-Smo Shmt1 -/-, Phgdh* f/f mice

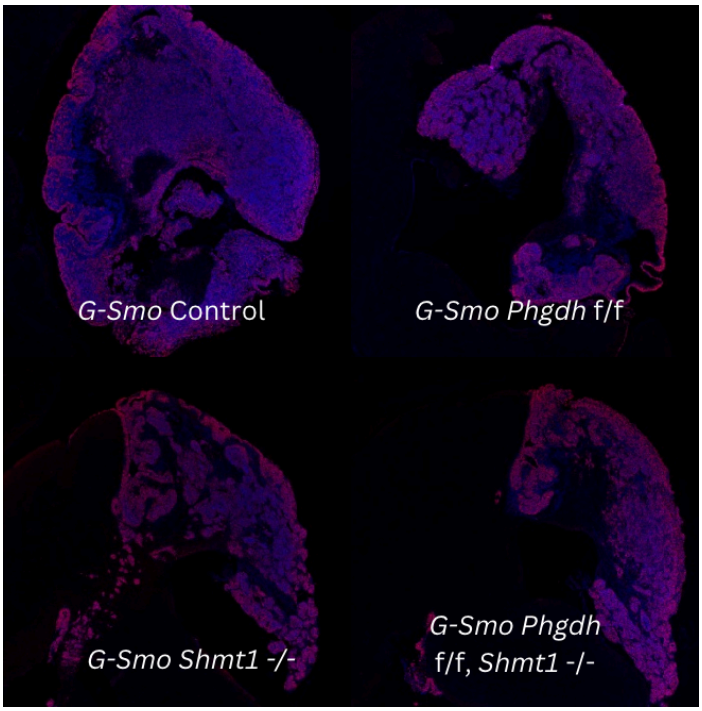

Figure 6: Proliferation staining in *Phgdh* and *Shmt1* deleted mice
Phospho-Rb IHC in tumors of *G-smo* Control, *G-Smo Phgdh* f/f, *G-Smo Shmt1* -/-, and *G-Smo Shmt1 -/-, Phgdh* f/f mice