

# Effect of DNA methyltransferase inhibition on human DIPG cell lines in hypoxic conditions

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Neuroscience

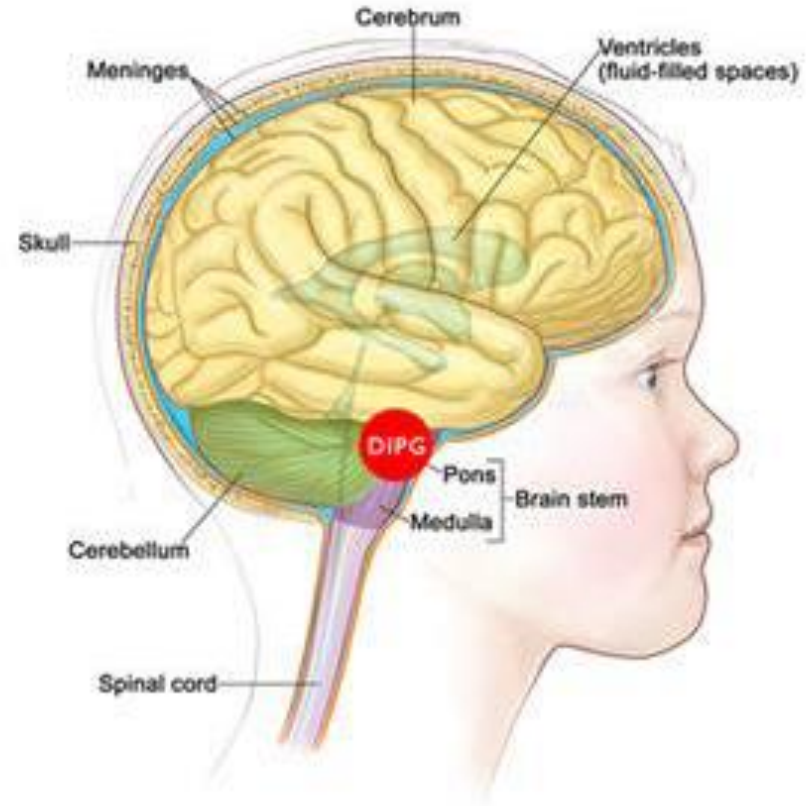
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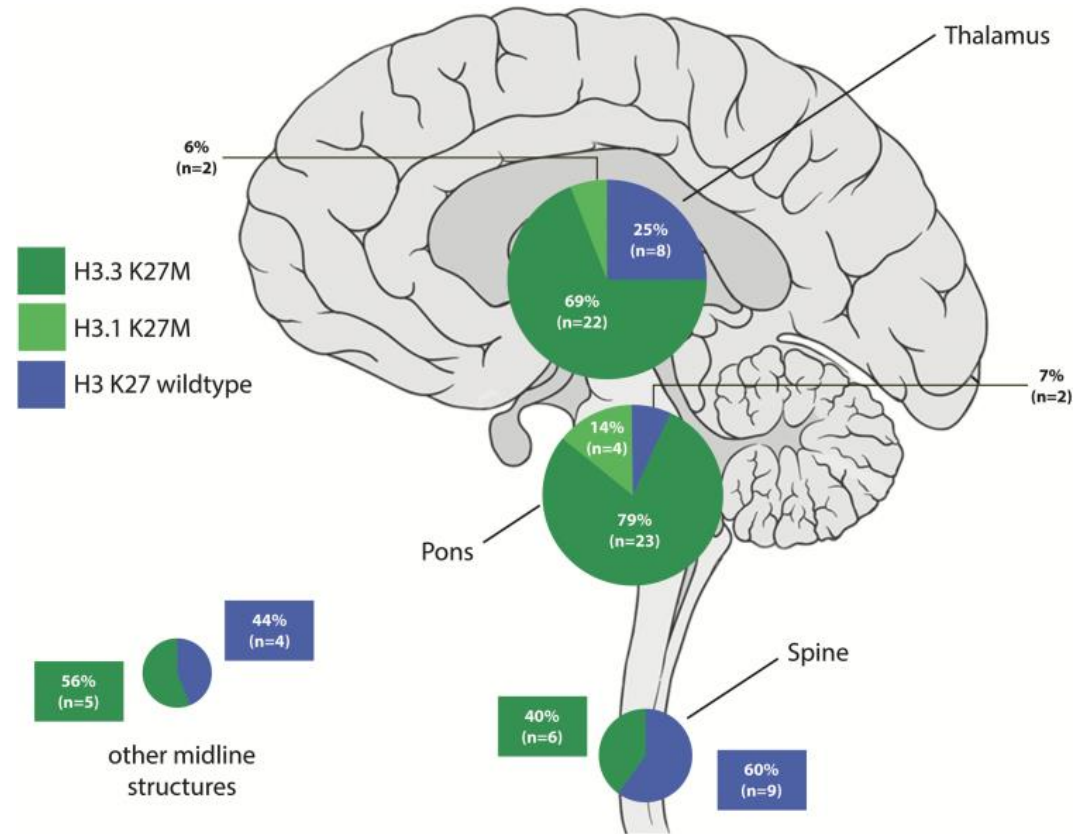
# DIPG- Diffuse Intrinsic Pontine Glioma

- Pediatric high grade glioma
- Constitutes 75% of brainstem malignancies found in children
- 5 year survival < 1%
- Limited treatment options
  - Surgically irresectable
  - Resistant to chemotherapy and radiation

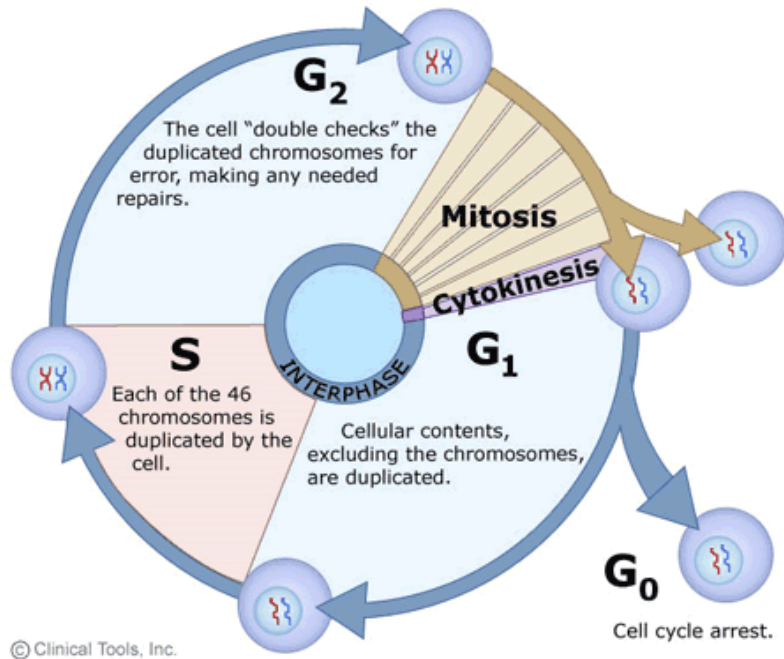


# H3.3K27M Mutation

- Most commonly observed mutation occurring in over 80% of DIPGs is a methionine substitution at lysine 27 on histone H3 (H3.3K27M)
- Results in observed global reduction of H3K27me2/3 and central gain of H3K27me3
- Dismal prognosis associated with H3.3K27M mutation is independent of location



# H3.3K27M-induced repression of p16/ink4A



**p16/ink4A**- a tumor suppressor and critical cell cycle regulator of the G<sub>0</sub>-G<sub>1</sub> to S-phase transition

Mechanism of H3.3K27M-induced repression of p16/ink4A had been proposed in which a DNA methyltransferase inhibitor rescued p16/ink4A expression

However, regions of **hypoxia** are evident within the solid tumor microenvironment of DIPGs and are associated with the distinct genetic profile and therapeutic resistance observed

# TKTL1

## **Transketolase-like 1 protein**

- Novel candidate oncogene largely associated with glucose metabolism
- Activated by promoter hypomethylation
- Accelerates aerobic glycolysis and HIF1 $\alpha$ -mediated tumor growth

**Overexpression shown to correlate with enhanced glycolysis and lactic acid production**

# Project Objectives

Investigate the overall cellular proliferation and epigenetic changes that occur upon treatment of human diffuse intrinsic pontine glioma cell lines with increasing concentrations of a DNA methyltransferase inhibitor, decitabine

Determine the effect of hypoxic conditions on drug efficacy and resultant expression in proteins of interest

In human DIPG cell lines, evaluate proposed mechanism of H3.3K27M-induced repression of p16/ink4A in which a DNA methyltransferase inhibitor rescued p16/ink4A expression, thus restoring cell cycle regulation and tumor suppression capabilities of p16 promoter

Further examine the impact of TKTL1, its role in DIPG tumor metabolism, and whether the increased expression observed previously upon decitabine treatment potentially plays a role in cellular adaptation to low oxygenated environments, or hypomethylated environments

# Methodology

## Western Blots:

- Whole cell lysates obtained from human DIPG tumor VUMC-DIPG-X
- One set of cultured cells treated in 10-fold increasing decitabine concentration starting at 0.001 to 10 micromolar for 24 hours, with DMSO treated vehicle
- Second set of cultured cells treated, same time, same concentrations, but in 24 hour pulse hypoxia (0.5% O<sub>2</sub>)
- Resolving gel, primary antibody concentration
- Followed standard Western Protocol

## Trypan Blue Assays

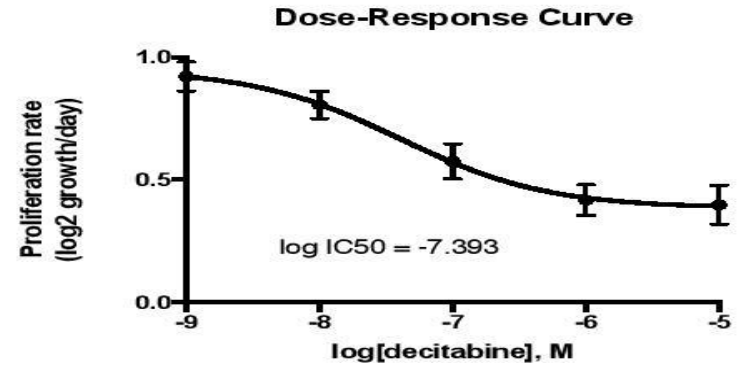
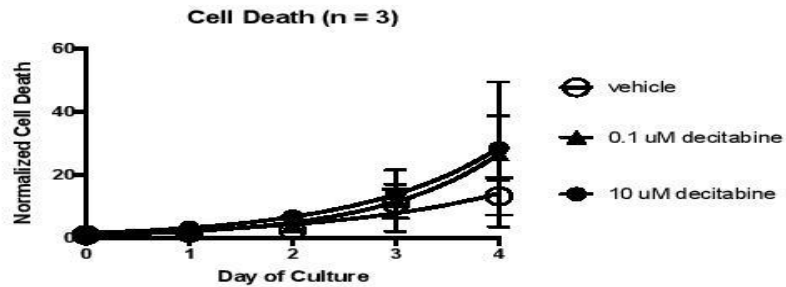
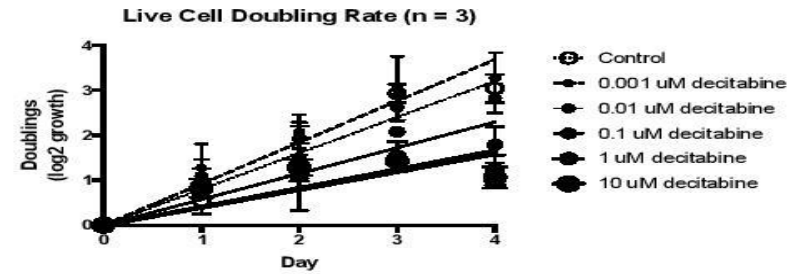
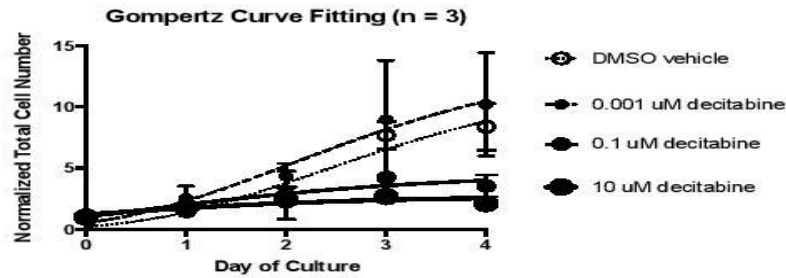
- Cultured human DIPG SU-DIPG-IV
- 6 well plates used, treated in 10-fold increasing decitabine concentration starting at 0.001 to 10 micromolar for 24 hours, with DMSO treated vehicle
- Cells were collected following standard Trypan Blue Assay Protocol and counted every 24 hours for 5 days using Invitrogen Automated Cell counter

# Results

\*due to COVID-19, results expected to be obtained were incomplete

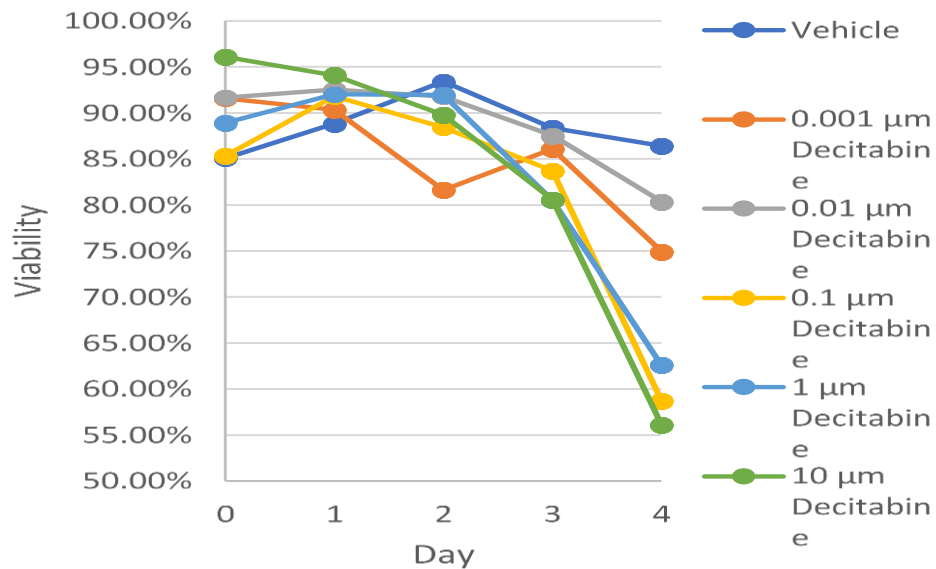


# SU-DIPG-IV- Decitabine 24 hour treatment normoxic conditions

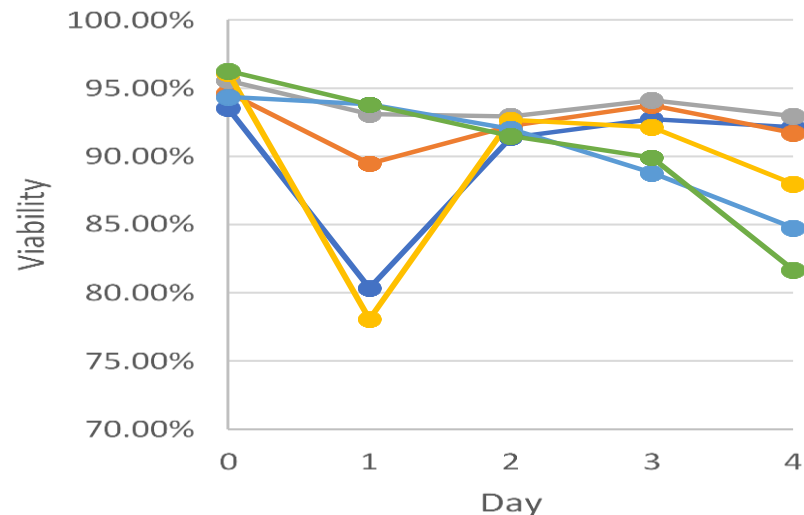


- Halted growth at highest concentration, warrants further investigation in to cell death mechanisms (apoptosis etc.)
- Upper limit of decitabine unknown, increased treatment concentration next step

## DIPG IV Decitabine Dose Response Normoxia Viability



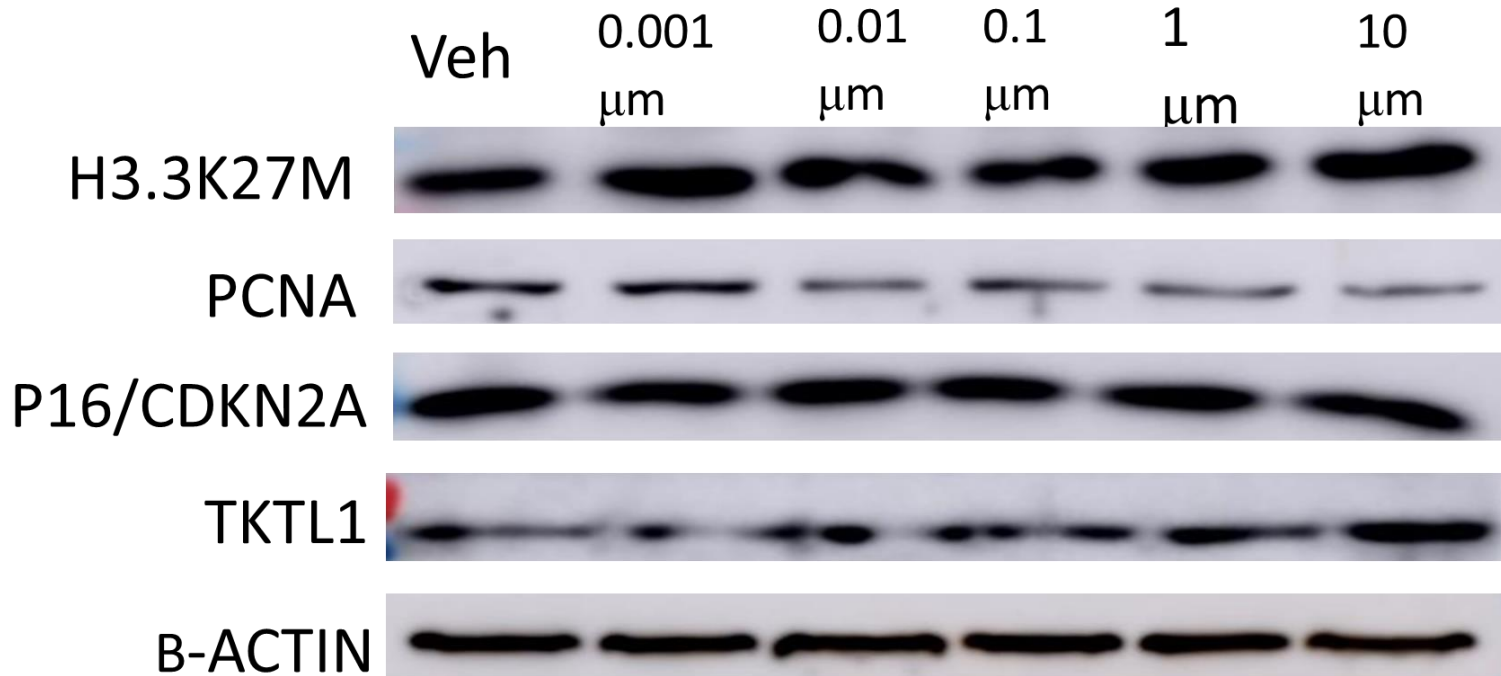
## DIPG IV Decitabine 24 Hr Pulse Hypoxia(0.5%) Dose Response Viability



Only was able to obtain a N=1 of DIPG IV normoxia and hypoxia viability comparison

## 24 Hr Decitabine-treated DIPG X normal O<sub>2</sub>

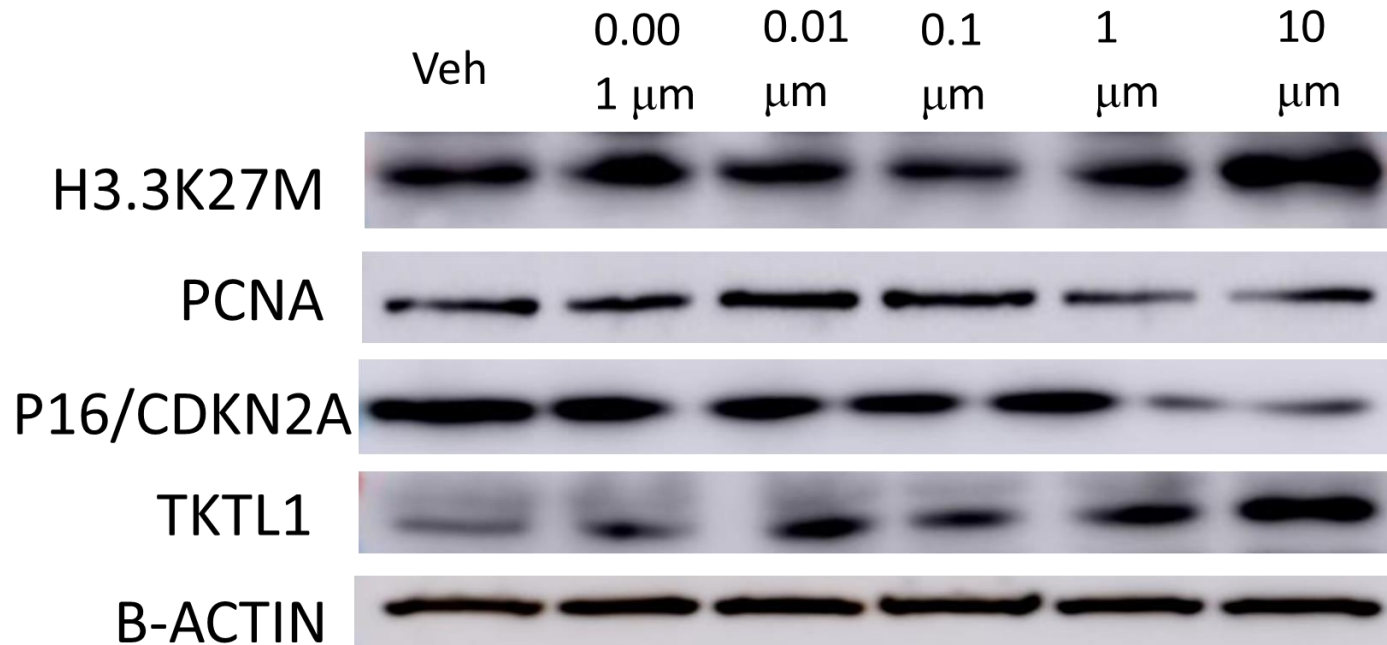
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- Decreased PCNA expression supports slowed cellular proliferation
- Increased TKTL1 expression

24 Hr Decitabine-treated DIPG X pulse-hypoxia  
treated (0.5% O<sub>2</sub> 24 hr)

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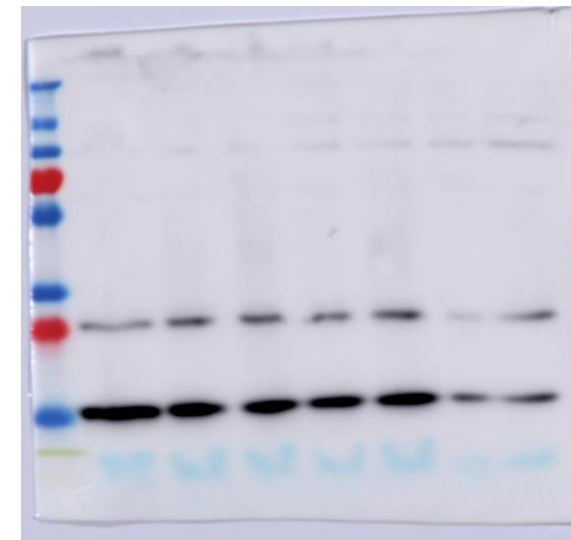


- Decreased PCNA expression supports slowed cellular proliferation
- A greater degree of TKTL1 expression, as expected with increased hypomethylation

# Interpretations- p16/CDKN2A

- p16 expression *appeared* to decrease with increasing decitabine concentration in hypoxic conditions, additional trials will follow to confirm, shown is a closer view of a p16 Western
- Whether or not the observed expression of p16 is considered repressed compared to normal brain tissue is yet to be determined and could be a possible explanation for the repression observed in prior studies

- DIPG X
- Decitabine 24 hour
- Hypoxia
- 10% resolving
- Primary 1:1000
- Secondary overnight 1:10000



Ladder	0M Decitabine Hypx	0.001 M Decitabine Hypx	0.01 M Decitabine Hypx	1 M Decitabine Hypx	10 M Decitabine Hypx

P16/CDKN2A 3.14.19

# Interpretations-TKTL1

- Increased expression of TKTL1 was observed in both normoxia and hypoxia treated cells with increasing decitabine concentration
- A greater increase of TKTL1 expression was observed in hypoxia-treated cells
- Suggests a possible role of TKTL1 in cellular adaptation to a low oxygen environment, thus offering a possible explanation for decreased efficacy of DNA methyltransferase inhibition observed in a hypoxic environment.

# Future Directions

- Further investigate the upper limit of decitabine treatment and determine the mechanisms of cell death following treatment using immunohistochemistry
- Further examine the role of TKTL1 in DIPG tumor metabolism, specifically hypoxic environments and activation of aerobic glycolysis

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