Hypoxia-inducible factors regulate DIPG growth in normoxic culture

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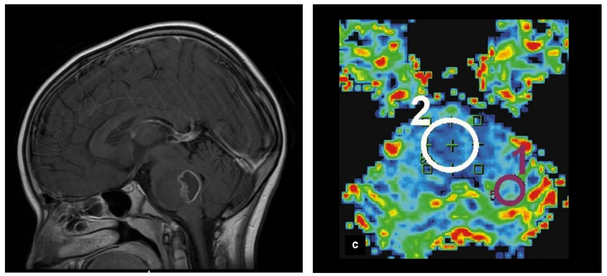
Abstract

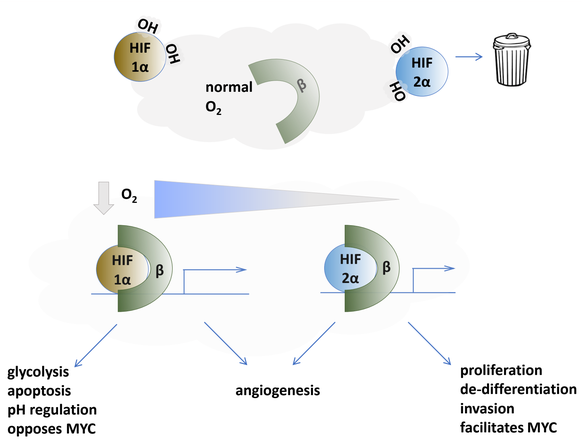
Diffuse intrinsic pontine glioma (DIPG) are incurable tumors and the leading cause of pediatric brain tumor deaths. They exhibit low blood perfusion and regions of necrosis, indicative of a low-oxygen environment that supports activation of hypoxia-inducible factors (HIF) that are associated with increased proliferation, invasion, and therapy resistance. However, previous reports suggest that HIF2-alpha slows growth in some glioma models. We therefore sought to test the hypothesis that HIFs regulate DIPG growth. We cultured the human DIPG tumors SU-DIPG-IV, VUMC-DIPG-X, and SU-DIPG-XIII at ambient oxygen tension and 5% carbon dioxide. We measured protein expression by Western blot and growth by trypan blue exclusion or tetrazolium reduction following exposure to the hypoxia-mimetic (HM) compounds, cobalt (II) chloride or deferoxamine, or selective HIF inhibitors. All three DIPG cultures retained stable expression of HIF1-alpha and HIF2-alpha protein at ambient oxygen tension, unchanged by HM treatment. Selective inhibition of HIF2-alpha by TC-S 7009 increased apparent growth, whereas selective inhibition of HIF1-alpha by CAY10585 did not. We conclude hypoxia-independent HIF expression unchanged by either HM treatment or HIF inhibition suggests impaired HIF degradation, in which hypoxia-induced activation of HIF target genes more likely depends on transcriptional co-activators rather than blocked proteasomal degradation. In both ambient and hypoxic conditions, HIF2-alpha activity may oppose DIPG growth. Future experiments will investigate whether the effects of HIF2-alpha inhibition on tumor growth can be explained by enhanced HIF1-alpha activity through desequestration of common binding partners, or through direct action of HIF2-alpha on previously reported apoptotic pathways.

Hypoxic microenvironment in DIPG

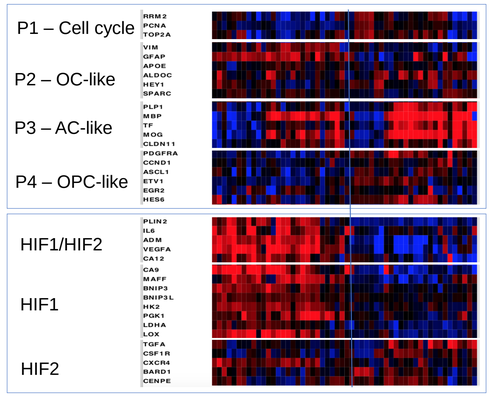


DIPG frequently show areas of necrosis, enhancement, and poor perfusion, suggestive of a hypoxic microenvironment. Right panel adapted from Yeom, Lober, *et al.*, 2015, *J Neurooncol* 122:383-389.

Differential effects of HIF isoforms



Some DIPG have increased HIF target expression



Heatmap from secondary analysis of H3 K27M brainstem gliomas (Mackay *et al.*, *Cancer Cell* 32:520-537) performed on ***PedcBioPortal*** (Cerami *et al.*, 2012, *Cancer Dis* 2:401; and Gao *et al.*, 2013 *Sci Signal* 6:pl1)

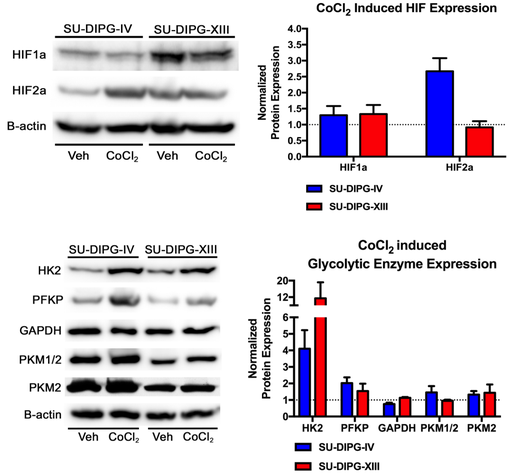
Tumor cell lineage gene expression programs recently identified in H3 K27M glioma subpopulations (Filbin *et al.*, 2018 *Science* 360:331-335) roughly correlate with HIF programs:

P1 : ↓ HIF1, ↑ HIF2
P2/3:↑ HIF1 or HIF2
P4: ↓ HIF1, ↑ HIF2

Cycling and stem-like cells (P1 and P4) appear to have more HIF2-only target expression and downregulated HIF1

Differentiated cells (OC- or AC-like) may have both.

Hypoxia and hypoxia-mimetics increase HIF target expression

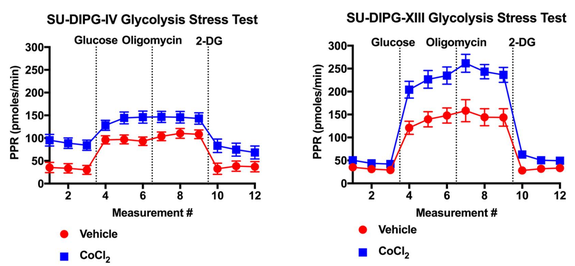


Western blot analysis of DIPG cultures treated with $CoCl_2$ demonstrate a large increase in HIF2 expression in SU-DIPG-IV and no change in SU-DIPG-XIII.

HIF1 target gene expression was assayed by Western blot analysis of Glycolysis enzymes. Increased expression of hexokinase II (HK2) and phosphofructokinase (PFKP) was observed with 24hr hypoxia mimetic treatment ($CoCl_2$). Minimal to no change were observed with phophokinase mutase (PKM) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Increased HK2 and HIF2 expression was observed with cultured DIPG treatment with 2% O_2 (not shown).

Hypoxia-mimetics increase DIPG glycolytic rate



Cells treated with 100uM $CoCl_2$ for 24 hr prior to assay.

Oligomycin inhibits ATP synthase.

2-DG inhibits hexokinase.

DIPG cultures exhibit increased glycolytic rate after treatment with $CoCl_2$ as compared to Control.

Acknowledgements

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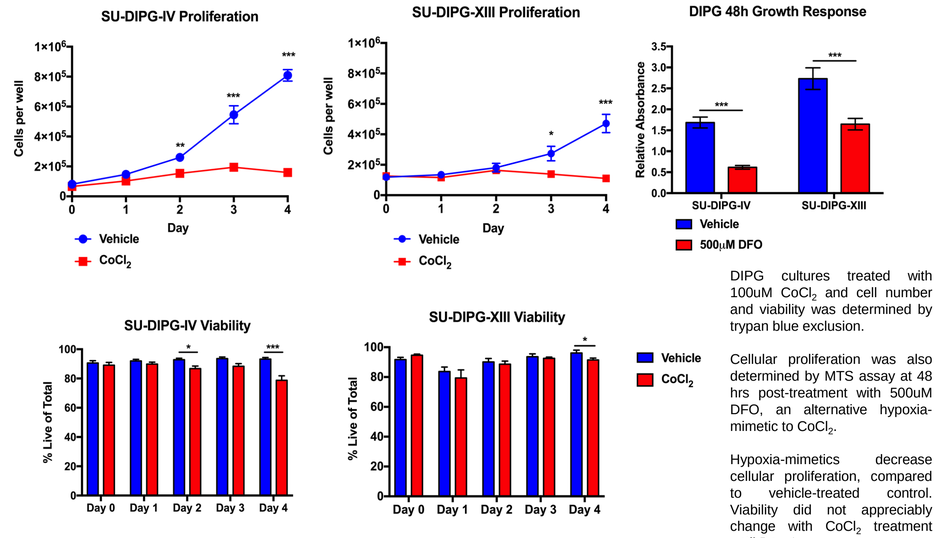
Cytostatic effect of hypoxia-mimetics on DIPG

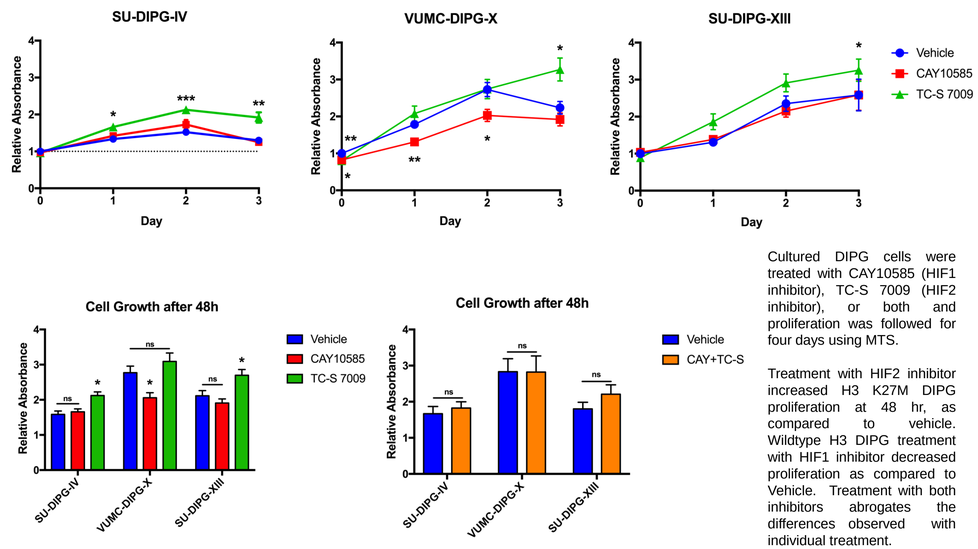


DIPG cultures treated with 100uM $CoCl_2$ and cell number and viability was determined by trypan blue exclusion.

Cellular proliferation was also determined by MTS assay at 48 hrs post-treatment with 500uM DFO, an alternative hypoxia-mimetic to $CoCl_2$.

Hypoxia-mimetics decrease cellular proliferation, compared to vehicle-treated control. Viability did not appreciably change with $CoCl_2$ treatment until Day 4.

HIF2 inhibitor treatment increases DIPG culture proliferation



Cultured DIPG cells were treated with CAY10585 (HIF1 inhibitor), TC-S 7009 (HIF2 inhibitor), or both and proliferation was followed for four days using MTS.

Treatment with HIF2 inhibitor increased H3 K27M DIPG proliferation at 48 hr, as compared to vehicle. Wildtype H3 DIPG treatment with HIF1 inhibitor decreased proliferation as compared to Vehicle. Treatment with both inhibitors abrogates the differences observed with individual treatment.

Conclusions and Future Directions

- Hypoxia-mimetics increase cultured DIPG glycolytic rate and enzyme expression, and decrease proliferation
- Hypoxia-inducible factors regulate cultured DIPG proliferation in ambient air conditions
- Future experiments will investigate the relationship of HIF1 and HIF2 to decreased proliferation in a lower-than-ambient oxygen tension.