Targeting Resistance Mechanisms in Medulloblastoma

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Abstract:

Background

Medulloblastoma is the most common malignant brain tumor in childhood. Although current therapeutic strategies have improved survival, they cause devastating and long-lasting sequelae and are inadequate in metastatic and recurrent disease where survival remains dismal. Effective therapies are therefore urgently needed to improve outcomes in this cancer.

The development of medulloblastoma is driven by dysregulation of normal cerebellar proliferation wherein aberrant sonic hedgehog (Shh) pathway signaling is often implicated and causes aggressive growth. Poor outcomes in Shh-driven tumors have prompted the evaluation of Shh-targeting agents in their treatment but these have had limited success - likely due to the upregulation of additional oncogenic pathways (e.g. Ras/MAPK and HIF-1α). These pathways stimulate glycolysis, in part by increasing the activity of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase family of enzymes (PFKFB1-4) to produce fructose-2,6-bisphosphate (F26BP), a potent activator of the rate-limiting glycolytic enzyme, 6-phosphofructo-1-kinase. We have recently found that the PFKFB4 family member is highly expressed in patient-derived Shh medulloblastomas.

Hypothesis and Results

We postulate that PFKFB4 is critical for medulloblastoma survival and growth and that PFKFB4 inhibition will significantly decrease their growth and metastasis.

We first confirmed PFKFB4 expression in Shh-driven medulloblastoma *in vitro* and found that hypoxia, through HIF-1α, strongly induced PFKFB4 in these cells. We then found that silencing PFKFB4 suppressed F26BP, glycolysis and proliferation in normoxia and more markedly in hypoxia, indicating that PFKFB4 may be required for growth in hypoxia. We found that co-silencing PFKFB4 and Shh proteins significantly reduced cell survival and that co-targeting PFKFB4 (with a novel small molecule inhibitor) and Shh effectors synergistically decreased cell viability. To simulate Shh-antagonist resistance, we subjected Shh medulloblastoma cells to prolonged Shh inhibitor exposure and have found that these cells show increased proliferation, glycolysis, anchorage independence and PFKFB4 and, additionally, higher sensitivity to PFKFB4 inhibition. We are now closely evaluating metabolic alterations in these cells.

Conclusions

Taken together, our data indicate that targeting PFKFB4 may be a valid therapeutic option in aggressive, treatment-resistant medulloblastoma and strongly support the examination of PFKFB4 inhibitors in these tumors.

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Figure 1. Highly aggressive medulloblastomas demonstrate oncogenic mutations that activate glucose metabolism. (A) Deregulation of the Shh pathway plays a critical role in medulloblastoma development. Shh binds to the patched receptor (PTCH) on granule neuron precursors to derepress Smo, activate the Gli transcription factors and target genes to promote proliferation and growth. (B) Aggressive medulloblastomas also demonstrate mutations of alternate pathways *e.g.* Ras/MAPK. This pathway increases glucose uptake and catabolism to lactate via multiple mechanisms including: (i) PI3K/AKT which activates the PFKFBs that in turn synthesize F26BP to activates phosphofructo-1-kinase (PFK1); (ii) Hypoxia Inducible Factor-1α (HIF-1α) which is found to promote expression of most glycolytic enzyme mRNAs; (iii) c-Myc which promotes expression of the Glut1 glucose transporter, PFK1, enolase (ENO) and lactate dehydrogenase (LDH); and (iv) PFKFB2-4 mRNA expression, promoted by HIF-1α.

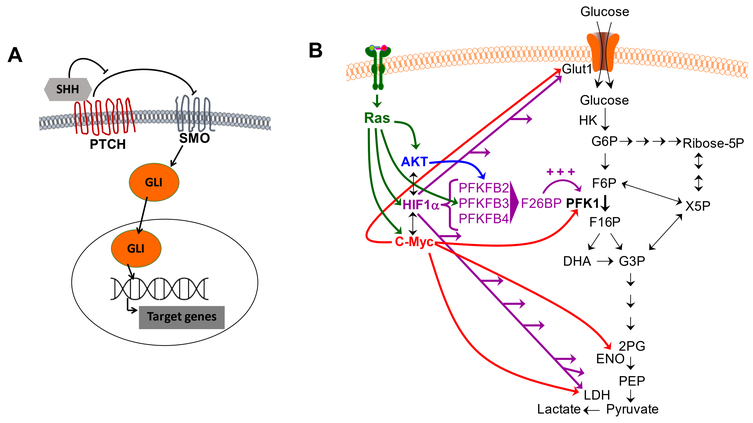


Figure 2. PFKFB4 expression is increased relative to other PFKFBs in medulloblastoma cell lines. PFKFB1-4 mRNA were quantified by realtime RT-PCR in normal human neural tissue and in Daoy (Shh) and D283 (Group 3/4) medulloblastoma cell lines (normalized to β-actin) and copy number calculated.

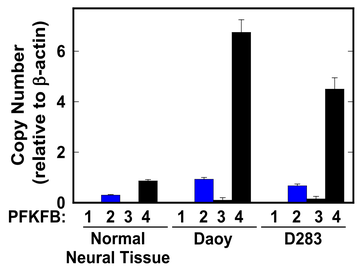


Figure 3. PFKFB4 expression is increased in screened patient-derived Shh medulloblastomas relative to adjacent normal tissue. (A) Medulloblastomas (T) and adjacent normal neural tissues (N) were examined by IHC for expression of PFKFB4 and the Shh effector protein Smoothened (Smo), representative sections shown; (B) positive pixels were quantified (Imagescope, Aperio) and (C) correlation between PFKFB4 and Smo was determined. * *p* value < 0.01.

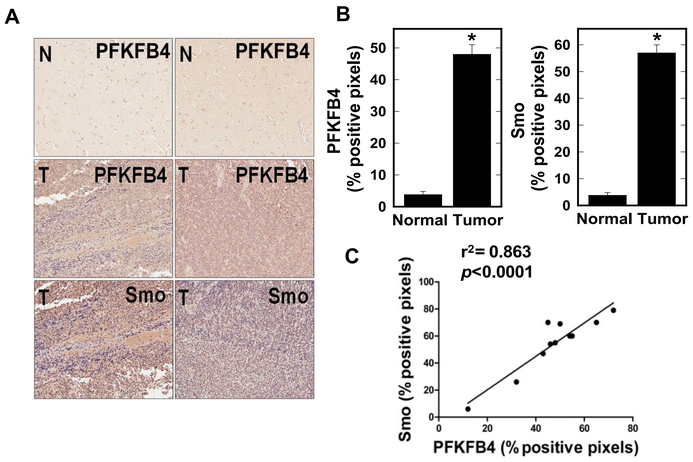


Figure 4. siRNA mediated silencing of PFKFB4 decreases F26BP and anchorage-dependent and independent growth in an Shh-driven medulloblastoma cell line. Daoy cells transfected with siRNA against PFKFB4 (siFB4) or scrambled siRNA (siCtrl) were exposed to 21% or 1% oxygen. Effects on PFKFB4 protein expression (β-actin, control) (A), [F26BP] (B) and live cell counts (C) were determined at 48 hours. Daoy cells transfected with siCtrl or siFB4 for 48 hours were plated in soft agar (n=3, 10^4 cells/plate). After 21 days, colonies were quantified (5 fields examined per plate), representative images shown (D). * *p* value < 0.01.

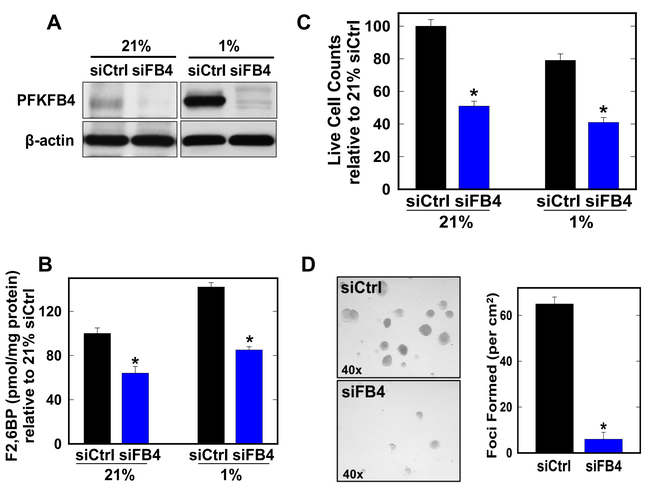


Figure 5. Co-targeting PFKFB4 and the Shh pathway synergistically decreases proliferation in Shh-driven medulloblastoma cells. (A) Daoy cells were transfected with control (siCtrl), PFKFB4 (siFB4), Smo (siSmo) or PFKFB4+Smo (siFB4/Smo) siRNA and viable cells were counted after 48 hrs. (B) Daoy cells were exposed to DMSO or increasing concentrations of a novel PFKFB4 inhibitor, FB4i ± a Smoothened inhibitor, GDC0449 (Vismodegib), and viable cells counted at 48 hrs. The combination index was calculated using Compusyn software. * *p* value < 0.01.

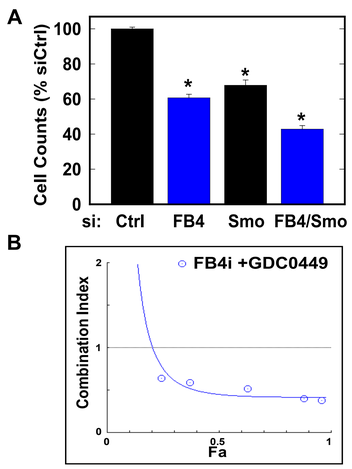
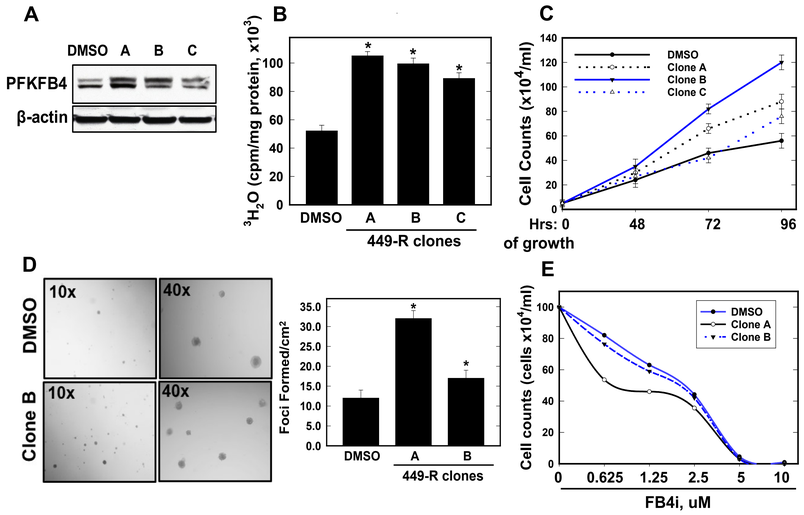


Figure 6. Shh-antagonist resistant medulloblastoma cells exhibit increased PFKFB4 expression, glycolysis and proliferation and sensitivity to PFKFB4 inhibition. Daoy cells were exposed to DMSO or increasing concentrations of an Shh antagonist for 12 months. DMSO-exposed cells and resistant clones were examined for (A) PFKFB4 expression; (B) glycolysis using 3H-glucose (*previously described, Chesney et al, Oncotarget, 2015)* and (C) proliferation at 0-96 hours. (D) Equal numbers of cells were plated in soft agar (n=3, 10^4 cells/plate) and after 15 days, colonies were quantified (5 fields examined per plate), representative images shown. (E) Clones were exposed to increasing concentrations of FB4i and viable cells counted at 48 hrs. * *p* value < 0.01.



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