Mutant IDH1 Suppresses Thrombosis in Gliomas

Laith Khoury MD; Steven Schwarze PhD; Thomas McIntyre PhD; Craig Horbinski BS, MD, PhD
Department of Pathology and Neurosurgery, University of Kentucky, Lexington, KY
Department of Pathology, New York University, New York, NY
Department of Cellular and Molecular Medicine, Cleveland Clinic Lerner Institute, Cleveland, OH

Introduction

Mutations in isocitrate dehydrogenase 1 and 2 (mutIDH1/2) are common in gliomas, resulting in enzymes that produce D-2-hydroxyglutarate (D-2-HG). The effects of mutIDH1/2 on the microenvironment, including thrombosis, are not known. Routine neuropathologic evaluation led to the discovery that IDH1/2-mutant gliomas do not usually contain micro thrombi

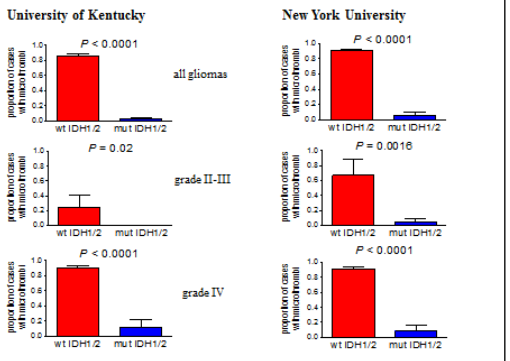

Figure 1. Microthrombi in gliomas.
In discovery (left) and validation (right) cohorts of WHO grade II-IV gliomas from two institutions (total N = 317), microthrombi were present in 85-90% of wild-type gliomas but in only 2-6% of mutant gliomas (P < 0.0001). This difference held across all glioma grades.

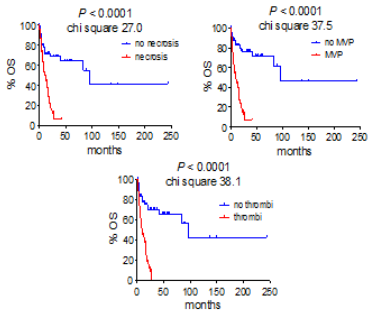

Figure 2. Univariate analysis of necrosis, MVP, and microthrombi for prognostic power.
On univariate analysis, the prognostic strength of microthrombi was comparable to necrosis and microvascular proliferation, the current WHO criteria for a diagnosis of glioblastoma. In fact, on multivariate analysis, microthrombi was superior to necrosis in prognostic strength.

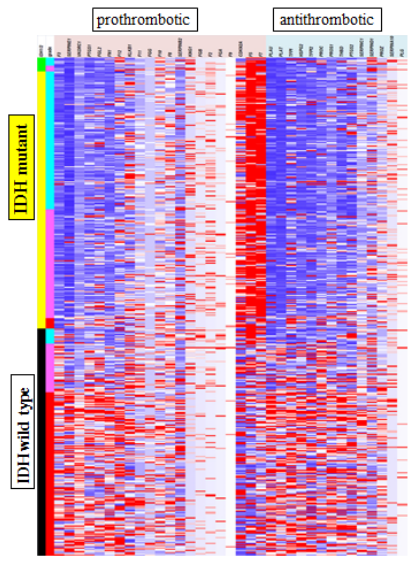

Figure 3. Glioma coagulome according to IDH mutation status.
Analysis of 428 gliomas from the Cancer Genome Atlas revealed that, in the coagulome, most coagulation genes are down regulated in gliomas and the mRNA with the strongest inverse relationship to mutIDH1/2 was F3, encoding tissue factor procoagulant. There was 75% less F3 mRNA in IDH mutant gliomas compared to wild-type gliomas ($P = 1.06 \times 10^{-52}$).

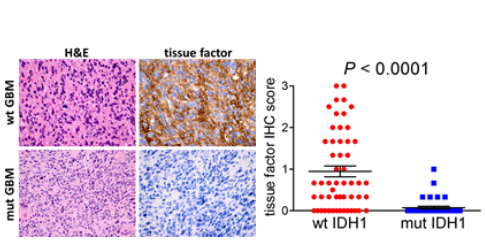

Figure 4. Glioma tissue microarray
Tissue factor expression by Immunohistochemistry. In a separate tissue microarray containing 95 gliomas, mutIDH1/2 tumors showed dramatically reduced TF protein expression (P < 0.0001).

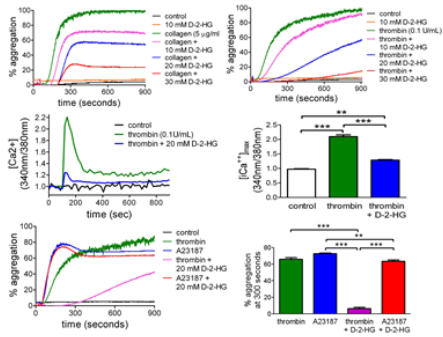

Figure 5. Platelet assay with D-2-HG and A23187
Top row, showing the effect of D-2-HG on platelet aggregation with increasing D-2-HG concentrations, as found in glioma microenvironment. Second row, showing the effect of D-2-HG on thrombin induced intracellular calcium spike. Third row, illustrates the changes in platelet aggregation when co-treatment with calcium ionophore, A23187.

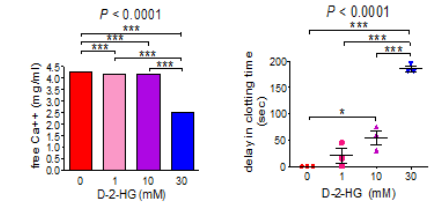

Figure 6. D-2-HG effect on local free Ca^{2+}
Left, showing at high concentrations, there is a drop in the free Ca^{2+} concentration. Right, showing that even at lower concentrations, there is a notable delay in clotting time.

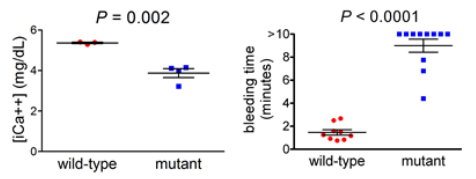

Figure 7. In vivo xenograft study in mice
Left, shows in mice with flank human glioma xenografts, those tumors expressing mutant IDH1 caused a 30% decrease in systemic serum free calcium. Right, shows bleeding time difference in wild-type vs mutant gliomas in a tail vein bleeding assay.

University of Kentucky (P<0.001)

DVT/PE /CVA	wt *IDH1/2*	mut *IDH1/2*
yes	30	0
no	88	47
total	118	47

New York University (P=0.0001)

DVT/PE /CVA	wt *IDH1/2*	mut *IDH1/2*
yes	31	0
no	74	31
total	105	31

Table 1. Systemic hypercoagulation and IDH1/2 mutations in gliomas.
Systemic hypercoagulation (including pulmonary embolus, deep venous thrombosis, or stroke) occurred in 30/118 (25%) and 31/105 (30%) of patients with wild-type gliomas from discovery and validation cohorts, respectively. In striking contrast, none of the 78 (0%) patients with mutIDH1/2 glioma developed systemic hypercoagulation in the combined discovery and validation cohorts.

Conclusions

- Mutant IDH1/2 enzymes have potent antithrombotic activities within gliomas and throughout the systemic circulation.
- This has profound implications for the pathologic evaluation of gliomas, the effect of altered metabolism on tumor microenvironment, and the postoperative management of these patients.
- Microthrombi are the most powerful predictor of IDH mutation and is an independant prognostic marker, justifying its inclusion in the WHO criteria for glioblastomas.