

# Mutant Isocitrate Dehydrogenase 1 Expression Enhances Response of Gliomas to the Histone Deacetylase Inhibitor Belinostat

Chi-Ming Chang <sup>1</sup>, Karthik K. Ramesh <sup>1,2</sup>, Vicki Huang <sup>1,2</sup>, Saumya Gurbani <sup>1</sup>, Lawrence R. Kleinberg <sup>3</sup>, Brent D. Weinberg <sup>4</sup>, Hyunsuk Shim <sup>1,2,4</sup> and Hui-Kuo G. Shu <sup>1,\*</sup>

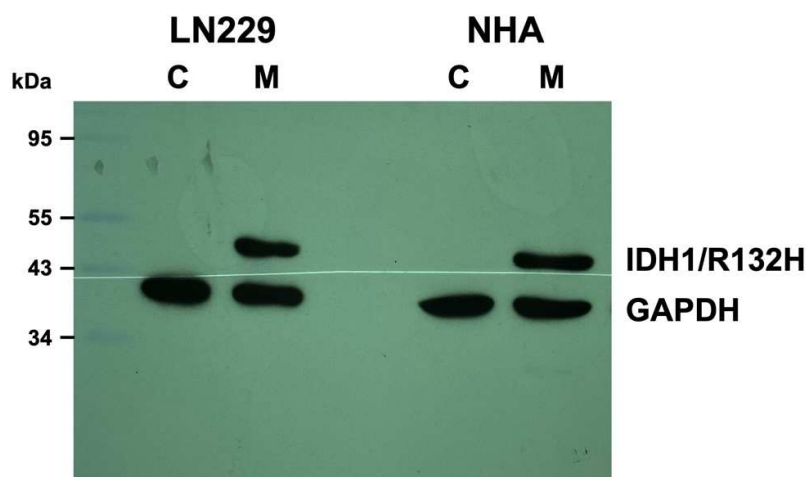
<sup>1</sup> Department of Radiation Oncology, Emory University, Atlanta, GA 30322, USA; chi-ming.chang@emory.edu (C.-M.C.); karthik.ramesh@emory.edu (K.K.R.); vicki.huang@emory.edu (V.H.); saumya.gurbani@emory.edu (S.G.); hshim@emory.edu (H.S.)

<sup>2</sup> Department of Biomedical Engineering, Emory University and Georgia Institute of Technology, Atlanta, GA 30322, USA

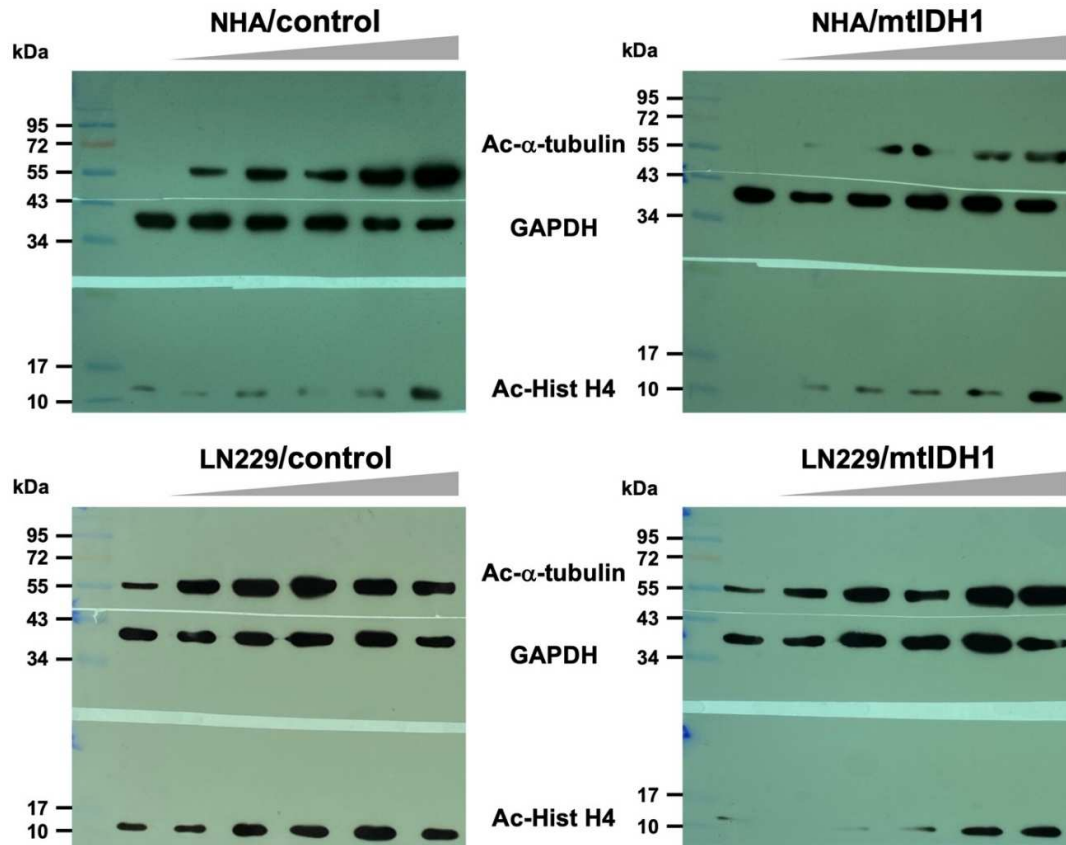
<sup>3</sup> Department of Radiation Oncology, Johns Hopkin University, Baltimore, MD 21287, USA; kleinla@jhmi.edu (L.R.K.)

<sup>4</sup> Department of Radiology and Imaging Sciences, Emory University, Atlanta, GA 30322, USA; brent.d.weinberg@emory.edu (B.D.W.)

\* Correspondence: hgshu@emory.edu; Tel.: +1-(404)-778-3473



**Figure S1.** The original western blot from Figure 1. Film detecting western blot is overlaid on cut filters with molecular weight marker lane shown on the left. Proteins that were probed for on each filter segment are indicated on the right. Note that GAPDH is the loading control on this blot.



**Figure S2.** The original Western blots from Figure 2 are shown with increasing  $\alpha$ -tubulin and histone H4 acetylation with increasing levels of the HDAC inhibitor belinostat indicated by the expanding gray triangles. Films detecting western blots are overlaid on cut filters with the molecular weight marker lane shown on the left side of each filter set. Proteins that were probed for on each filter segment are indicated between the blots.

**Table S1.** Normalized densitometry values of bands from western blots.

Figure 1	NHA/C		NHA/M		LN229/C		LN229/M	
mtlDH1	0.01		0.73		0.02		0.68	

Figure 2	NHA/C		NHA/M		LN229/C		LN229/M	
Belinostat ( $\mu$ M)	Ac-tub	Ac-H4	Ac-tub	Ac-H4	Ac-tub	Ac-H4	Ac-tub	Ac-H4
0.0	0.01	0.15	0.03	0.07	0.53	0.70	0.62	0.22
0.1	0.33	0.05	0.17	0.01	1.38	0.76	1.04	0.00
0.3	0.60	0.24	0.60	0.09	1.45	1.05	1.01	0.06
1.0	0.56	0.09	0.57	0.36	1.37	0.97	0.67	0.26
3.0	1.25	0.30	0.94	1.23	1.08	1.21	1.02	0.61
10.0	1.80	1.03	1.28	1.44	1.27	1.32	1.81	1.10

**Abbreviations:** NHA: normal human astrocytes; C, control; M, mutant IDH1-expressing; mtlDH1, IDH1 R132H mutant; Ac-tub, acetylated- $\alpha$ -tubulin; Ac-H4, acetylated-histone H4.

**Methodology:** Densitometry values were determined by scanning films of the relevant western blot on a standard flatbed scanner detecting the blots as 8-bit grayscale images. Images were loaded into Image J (available at <https://imagej.net/ij/index.html>, accessed on 26 April, 2023) and densitometry values for the bands of interest (mtlDH1, Ac-tub, Ac-H4) as well as loading controls (GAPDH) were determined by standard techniques. Each normalized densitometry value was determined by dividing the densitometry value of the band of interest by the densitometry value of the corresponding loading control.