IDH mutated tumors promote epileptogenesis via D-2-HG dependent mTOR hyperactivation

Armin Mortazavi, BS; Islam Fayed, MD; Muzna Bachani, BS; Alex Ksendzovsky, MD, PhD; Dragan Maric, PhD; Tyrone Dowdy, PhD; Mioara Larion, PhD; Chun Zhang Yang, PhD; Kareem A. Zaghloul, MD, PhD;

FUNCTIONAL AND RESTORATIVE NEUROSURGERY UNIT

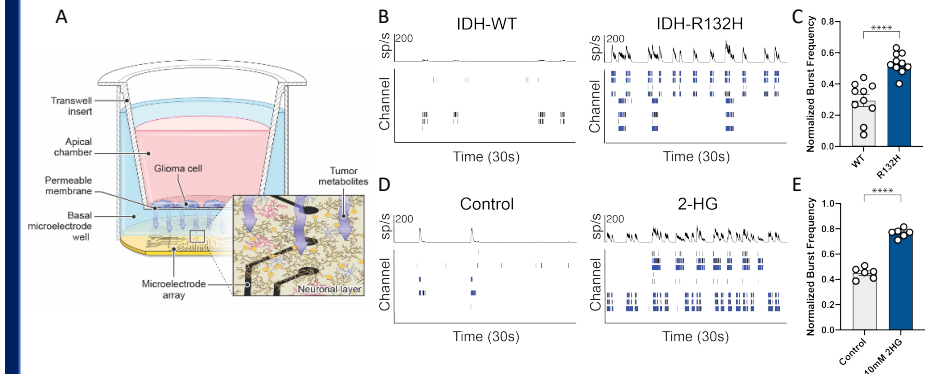
Introduction

Epileptic seizures in patients with low-grade, isocitrate dehydrogenase (IDH) mutated gliomas reach 90%, a major source of morbidity for these patients. Albeit there are multiple features that contribute to tumor related epileptogenesis, IDH mutations are determined to be an independent factor, although the pathogenesis remains poorly understood. We demonstrate IDH-mutated tumors promote epileptogenesis through D-2-hydroxyglutarate (D-2-HG) dependent mTOR hyperactivation and metabolic reprogramming.

Methods

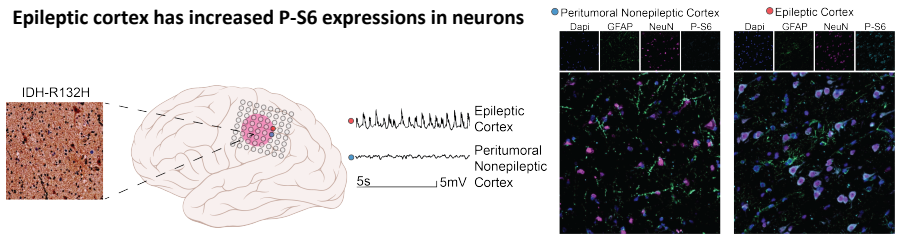
Human epileptic and nonepileptic cortex were identified via subdural electrodes in patients with IDH-mutated gliomas (n=5). An *in vitro* rat cortical neuronal model on microelectrode arrays were utilized to investigate the role of D-2-HG on neuronal excitability. mTOR and lysine demethylase (KDM) modulators were applied to elucidate the epileptogenic mechanism. Tetrodotoxin was utilized to evaluate the contribution of neuronal activity to mTOR signaling and metabolism. mTOR signaling was evaluated through western blot analysis and multiplex immunofluorescence. Metabolic function were analyzed via Seahorse assays and metabolomic analysis.

IDH mutated tumors promote epileptogenesis via D-2-HG



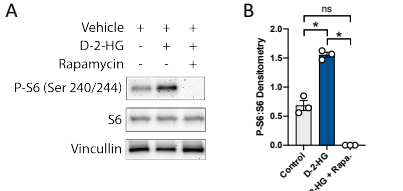
A) Schematic of transwell model allowing communication between glioma cell line (IDH WT or R132H) and cortical rat neurons cultured on a microelectrode array (MEA). B) Thirty second raster plots (bottom) and spike histograms (top) of spiking activity in eight electrode channels in a single well with cortical rat neurons. IDH^{R132H} (right) induced greater number of bursts (*blue bars*) compared to IDH^{WT}. C) Normalized burst frequency across 10 biological replicates demonstrating increased bursting activity of neurons interacting with IDH^{R132H} compared to IDH^{WT} (n=10, mean ± SEM, **** p<0.0001, paired t-test). D-E) D-2-HG induced greater bursting activity compared to control (n=6, mean ± SEM, **** p<0.0001, paired t-test).

Epileptic cortex has increased P-S6 expressions in neurons



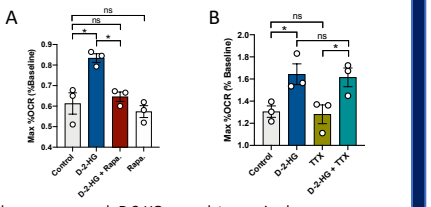
A) Diagram demonstrating identification of epileptic cortex in the setting of IDH mutated tumor. B) Multiplex immunofluorescence staining of Dapi, GFAP (astrocyte marker), NeuN (neuronal marker), and P-S6 (marker of mTOR activation) demonstrating increased mTOR activity in neurons within epileptic cortex compared to nonepileptic cortex.

D-2-HG results in mTOR hyperactivation



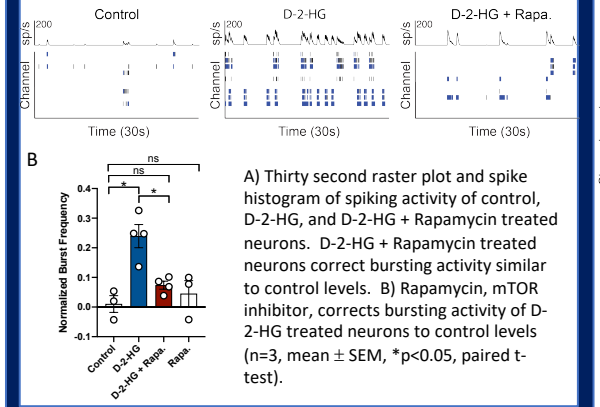
A-B) Western blot analysis reveals D-2-HG upregulates mTOR hyperactivation in cortical neurons, which is inhibited with rapamycin (n=3, mean ± SEM, *p<0.05, paired-t test).

D-2-HG increases OCR in an mTOR dependent



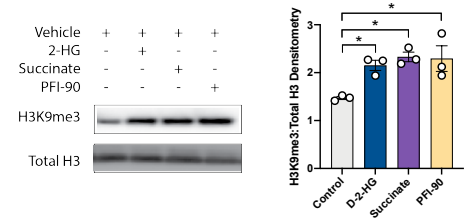
A-B) Seahorse assay reveals D-2-HG upregulates maximal oxygen consumption rate (OCR) that is reversed with mTOR inhibition (A), but is independent of neuronal firing (B) (n=3, mean ± SEM, *p<0.05, paired-t test).

mTOR inhibition corrects epileptogenic activity



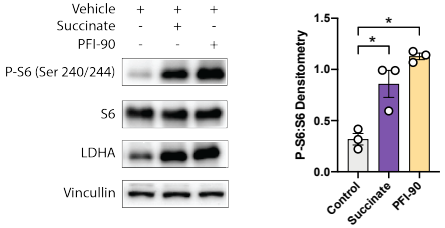
A) Thirty second raster plot and spike histogram of spiking activity of control, D-2-HG, and D-2-HG + Rapamycin treated neurons. D-2-HG + Rapamycin treated neurons correct bursting activity similar to control levels. B) Rapamycin, mTOR inhibitor, corrects bursting activity of D-2-HG treated neurons to control levels (n=3, mean ± SEM, *p<0.05, paired t-test).

D-2-HG inhibits KDMs



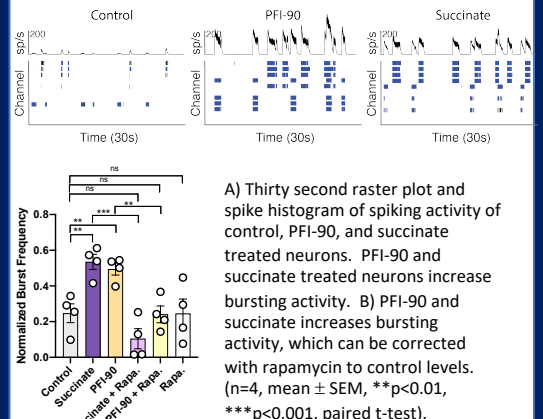
A) Western blot analysis of trimethylated Histone H3K9 and total Histone 3 demonstrating increased methylation, secondary to lysine demethylase inhibition.

KDM inhibition upregulates mTOR signaling



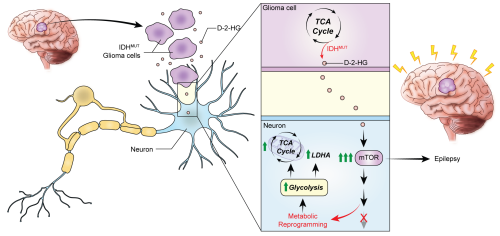
A-B) Western blot analysis reveals Succinate and PFI-90, KDM inhibitors, upregulate mTOR signaling (n=3, mean ± SEM, *p<0.05, paired-t test). KDM inhibition results in mTOR activation.

KDM inhibition upregulates mTOR signaling, resulting in neuronal hyperactivity that can be corrected with mTOR inhibition



A) Thirty second raster plot and spike histogram of spiking activity of control, PFI-90, and succinate treated neurons. PFI-90 and succinate treated neurons increase bursting activity. B) PFI-90 and succinate increases bursting activity, which can be corrected with rapamycin to control levels. (n=4, mean ± SEM, **p<0.01, ***p<0.001, paired t-test).

IDH mutated tumors promote epileptogenesis via D-2-HG induced mTOR hyperactivation



We propose IDH mutated tumors secrete D-2-HG to the peritumoral environment activating mTOR signaling in the human cortex, which results in metabolic reprogramming and neuronal hyperactivity.

Conclusions:

- IDH mutated tumors promote epileptogenesis via D-2-HG
- D-2-HG upregulates mTOR signaling via KDM inhibition, which promotes metabolic reprogramming and neuronal hyperactivity
- mTOR inhibition corrects neuronal hyperactivity and metabolic reprogramming