TET Activation Decreases Post-Stroke Cell Death

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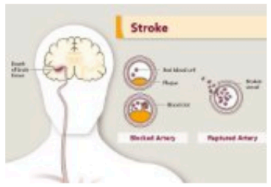
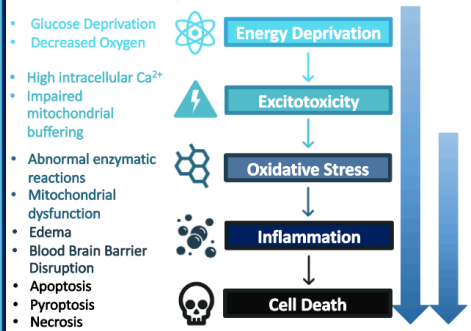
Background

Ischemic Stroke

Ischemic stroke is a neurological condition resulting in loss of blood flow in the brain due to a blood clot or atherosclerosis. Current therapies for stroke are severely limited, thus the development of new treatments is warranted.

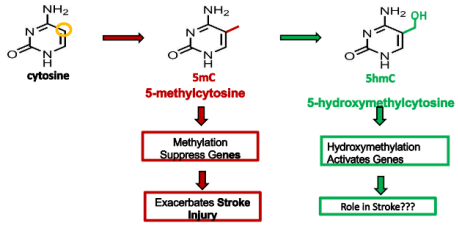


Ischemic Stroke Pathophysiology

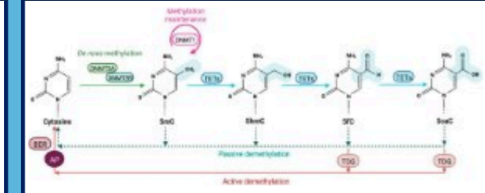


Epigenetics

Epigenetics is the study of how behavioral and environmental stressors affect the way genes are expressed without changing the genetic sequence itself. 5-methylcytosine (5mc) is one such example of an epigenetic modulation to DNA that has been experimentally shown to increase after stroke. These findings also indicate that an abundance of 5mc inhibits gene expression, leading to poorer outcomes after stroke. Ten-eleven translocase (TET) enzymes are epigenetic modifiers that convert 5mc to 5-hydroxymethylcytosine (5hmc). Though the role of 5hmc is poorly understood, emerging evidence has indicated it plays a neuroprotective role.



Approach



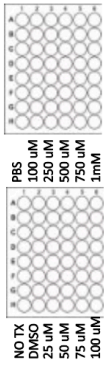
Objectives:

- How does OGD impact cell death in HT-22 hippocampal cells?
- Does pharmacological TET activation correlate to less overall cell death in HT-22 hippocampal cells?
- Does pharmacological TET inhibition correlate to more overall cell death in HT-22 hippocampal cells?

TET Activation

Ascorbate is the form of vitamin C which is required for many essential metabolic reactions in humans. It is known to activate TET enzymes.

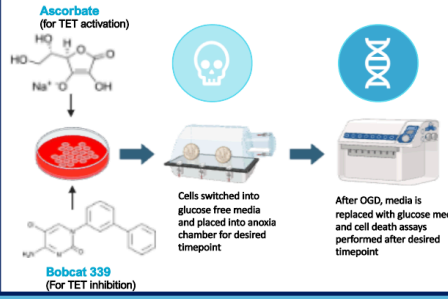
- Treat 96 well plates with varying concentrations of ascorbate, subject them to OGD, and then assess cell death.
- Treat 100 mm dishes with varying concentrations of ascorbate, subject them to OGD, collect cells, and perform dot blots to assess relative amount of methylation and hydroxymethylation



TET Inhibition

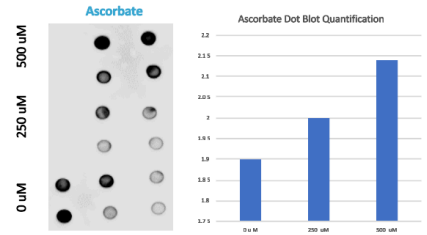
Bobcat 339 is a synthetic cytosine-based TET inhibitor. Bobcat is soluble in DMSO.

- Treat 96 well plates with varying concentrations of ascorbate, subject them to OGD, and then assess cell death.
- Treat 100 mm dishes with varying concentrations of ascorbate, subject them to OGD, collect cells, and perform dot blots to assess relative amount of methylation and hydroxymethylation



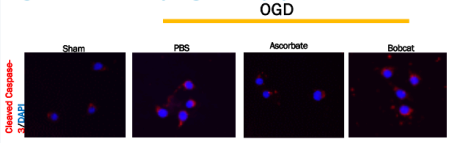
In Vitro Results

Figure 1: Ascorbate Treatment Modulates 5hmc



Dura
1:500 Primary
1:5000 Secondary
1% milk

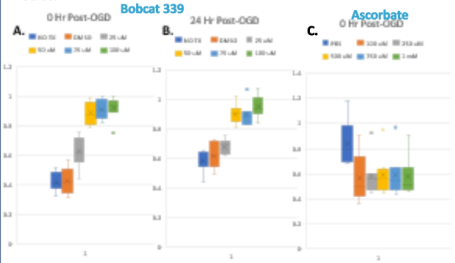
Figure 2: TET Activity Regulates Apoptosis After OGD



OGD performed on coverslip wells and then imaged for TET activity post-OGD using immunofluorescent detector.

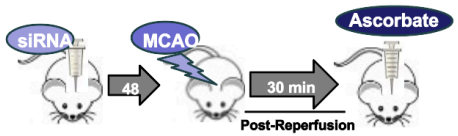
Figure 3: TET Activity Regulates Necrosis After OGD

OGD performed at AB) 6 hours and C) 12 hours. Cell death was then assessed immediately post-OGD and 24 hours post-OGD using propidium iodide.

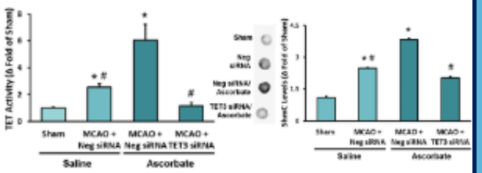


In Vivo Results

Figure 4: TET Activity Prevents Brain Damage in Vivo

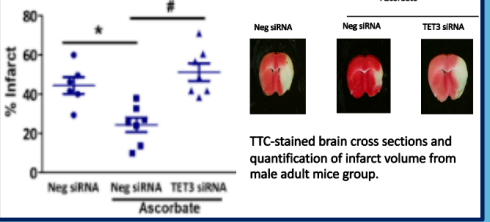


Mice administered 48 hour pretreatment of TET3 siRNA before middle cerebral artery occlusion (MCAO) was performed. After 30 minutes of reperfusion, mice were injected with ascorbate at 500 mg/kg



A) TET catalytic activity in nuclear lysates isolated at 6h reperfusion following transient MCAO and B) dot blot quantification of 24 hour-post-reperfusion

Ascorbate Protects Against Secondary Brain Damage Post-Stroke



TTC-stained brain cross sections and quantification of infarct volume from male adult mice group.

Conclusions

- OGD increases cell death in HT-22 hippocampal cells.
- Pharmacological TET activation correlates to less overall cell death in HT-22 hippocampal cells
- Pharmacological TET inhibition correlates to more overall cell death in HT-22 hippocampal cells

Acknowledgements

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References

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