Twisting Our Understanding of Glioblastomas

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Gliomas

- Glioma – tumor from glia brain tissue
- Glioblastomas (GBM) - Grade IV astrocytoma
- Highly malignant
- Deadly
- Glioma Stem Cells
  - Responsible for invasive potential and recurrence of GBMs
What is Twist1?

Twist1 (Transcription factor)

Dimerization

Transcription activator as suppressor

Development:
- Regulates mesodermal development

Tumor:
- Regulator of Epithelial Mesenchymal Transition (EMT)
- Promotes tumor cell metastasis
Experimental Plan

Literature: Decrease in Twist1 decreases invasive properties of malignant cells

Rostomily Lab: Twist1 also affects the ability to form a tumor

Hypothesis:

If Twist1 is deleted from human glioma cells then it will decrease tumorigenicity
How to Determine Twist1 Function in Glioma

Loss of Function:
- Cre/Lox Technology
- siRNA
- CRISPR/Cas9

Gain of Function
- Gene overexpression
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
Twist deletion may reduce tumor malignancy

Survival of U87NT vs dTwistA

P = 0.0004

Cas9/Flag
TW
β-Actin

U87NT
U87dTW-A
Twist+ uninfected cells are selected for in mouse tumor formation
Detection of Cas9 Expression in U87MG Derived Tumors Growing in Mouse Brain

Control
U87NTFlag

Red = positive for Cas9

U87dTwistA

No expression of Cas9
What happened?

**Expected Results**

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<td>Twist +</td>
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<td>Twist -</td>
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**Experimental Results**

**Injected Cells** → **Resulting Tumor**
Conclusion

• U87 cells have significantly reduced ability to form tumors (significantly decreased tumorigenicity)

• Next Experiment: Isolate U87 dTwistA single cell sub-clones that do not have contaminants (absolutely no twist expression)
  • Isolate single cell clones
  • Perform screening for presence of mutations
    • Surveyor assay and direct sequencing
    • Determine which InDels generate pre-mature stop codon and remove any WT or twist revertant cells
U87 subclone PCR

- 1kb Ladder
- U87 sub-clone 7
- U87 sub-clone 8
- U87 sub-clone 9
- U87 sub-clone 11
- U87 sub-clone 13

737bp
Deletion of Cas9

- 1 kb Ladder
- dTW-A
- XbaI+BamHI

Cas9
Out of Lab Activities

- Tumor Board Meetings at UWMC
- Neurosurgery Conference at UWMC
- Gamma Knife at HMC
- Neurological Surgery Resident Shadowing
- Awake Craniotomy
- Bifrontal Craniotomy
- Pediatric Neurosurgery
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