

**Internship presentation:**

**Impact of age on tumor malignancy/TWIST1  
promotes invasion through mesenchymal  
change in human glioblastoma**

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# Rostomily lab introduction

- Focus on molecular biology of brain tumor stem cells
- Mechanisms directing age related **malignancy and invasion**
- Large focus on clinical applications; surgical background
- *Goal:* develop better treatments through research based on connections with “developmental and aging neural stem and progenitor cell biology and human brain cancers”

# Impact of age on tumor malignancy: Theory/Hypothesis

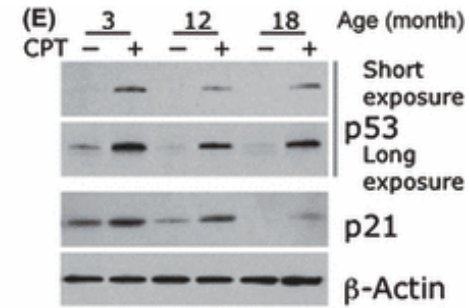
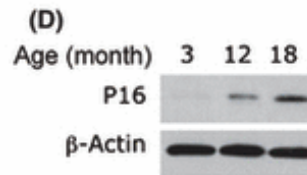
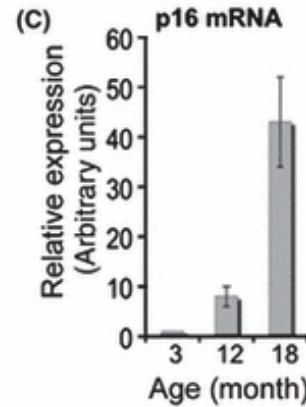
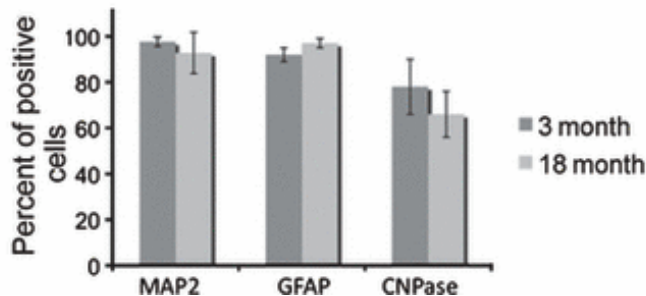
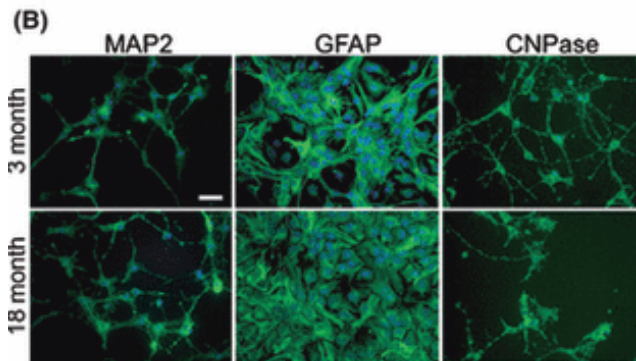
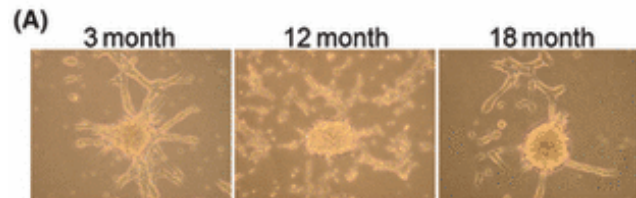
- *Neural progenitor cells (NPCs)*- presumed cells of glioma origin
- *Malignancy*- tendency to progressively worsen/grow and lead to patient death (measured by cell proliferation rates and survival rates)
- What is the relationship between age and tumor malignancy in patients?
- Hypothesis: Incidence, malignancy, and treatment resistance are correlated to patient age; directly proportional
- ...the age of NPCs influences glioma malignancy

# Impact of age on tumor malignancy:

## Methods/Results

- **TEST 1: 3-, 12- and 18-month old neural progenitor cells** were used to generate gliomas, which were then placed into **the same aged adult hosts**
- **TEST 2:** Implanted GL261 glioma cells into syngeneic 3- and 18-month old host C57B1/6 mice (**same aged cells placed into different aged hosts**)
- **RESULTS 1:** 18-month old NPCs had median survival of 38.5das, 12-month old NPCs had median survival of 42.5days and the 3-month old NPCs had median survival of 77days
- *...the gliomas generated from the older (18mos) NPCs caused the host's death significantly faster than those generated from younger NPCs (3mos or 12mos)*
- **RESULTS 2:** no difference in survival
- *...the difference in host age is not statistically significant*
- Confirmed that p16 tumor suppressor expression increased with NPC age

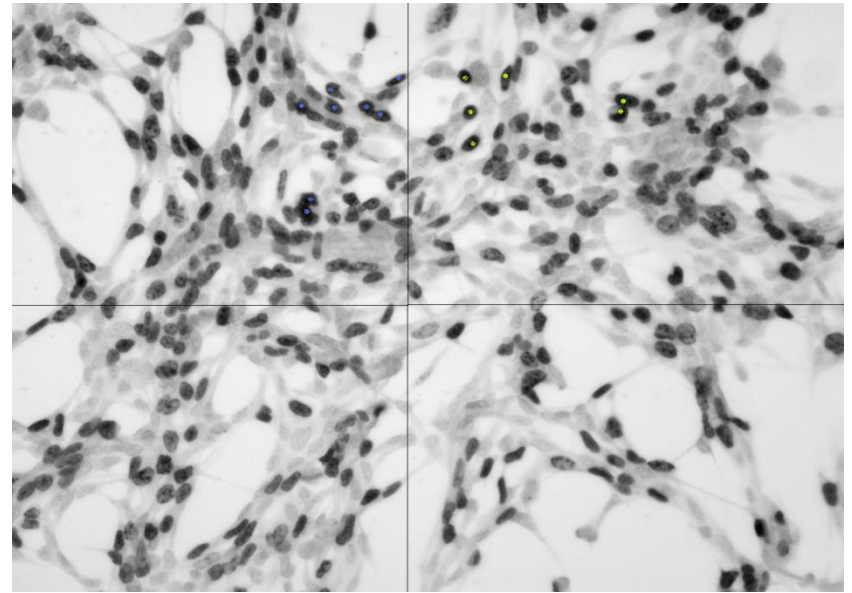
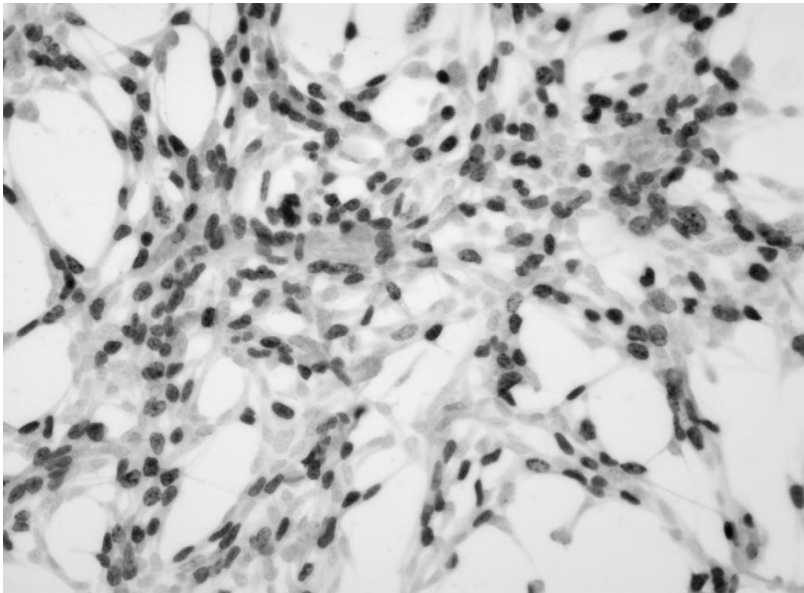
# Figure 1: Isolation and characterization of neural progenitor cells (NPC) from different-aged donor brain



# Impact of age on tumor malignancy:

## Personal contribution

- Culture cells (split cells, feed cells with growth factors, count cells, calculate volumes/concentrations)
- “BrdU” labeling & immunocytochemistry protocol
- Staining cells/positive vs. negative... identify cell proliferation
- Photograph cells (a special thanks to Dr. Richard Morrison)
- Count cells
- Calculate differences



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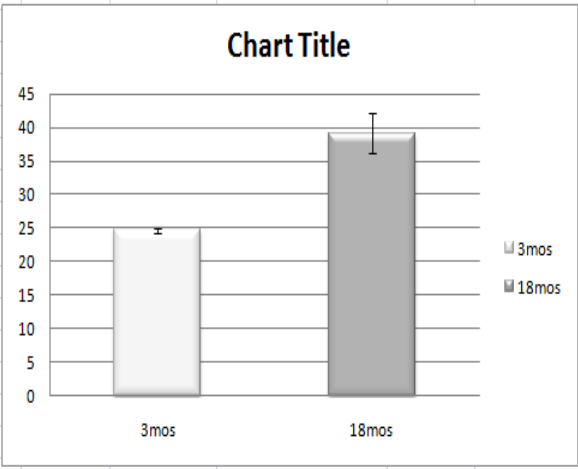
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General

Normal Bad Good Neutral Calculation Check Cell

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
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56	Well 4, Image 3, Box 1	57	8	14.04	77	27	35.06										
57	Well 4, Image 3, Box 2	88	29	32.95	88	33	37.5										
58	Well 4, Image 3, Box 3	64	17	26.56	91	31	34.06										
59	Well 4, Image 3, Box 4	60	18	30	68	24	35.29										
60	Total Well 4, Image 3	269	72	26.77	324	115	35.49										
61	Well 4, Image 4, Box 1	68	19	27.94	87	32	36.78										
62	Well 4, Image 4, Box 2	117	44	37.6	53	18	33.96										
63	Well 4, Image 4, Box 3	72	23	31.94	83	32	38.55										
64	Well 4, Image 4, Box 4	107	43	40.18	51	22	43.14	3 Months	18 Months	3 Months	18 Months						
65	Total Well 4, Image 4	364	129	35.44	274	104	37.95	Average	Average	Standard Deviation	Standard Deviation						
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# TWIST1 promotes invasion through mesenchymal change in human glioblastoma:

## Theory

- “Tumor cell invasion into adjacent normal brain” is a characteristic of GBM
- Restricts curative resection
- Limits effectiveness of chemotherapy and radiation
- Carcinoma invasion and metastasis are driven by EMT
- TWIST1 is a transcription factor that contributes to regulation of cell migration, reduced adhesion...
- ...previously established that TWIST1 is up-regulated in malignant gliomas and promotes invasion *in vitro*
- Targeting TWIST1-mediated mesenchymal change could potentially inhibit GBM invasion and tumor growth... better treatments

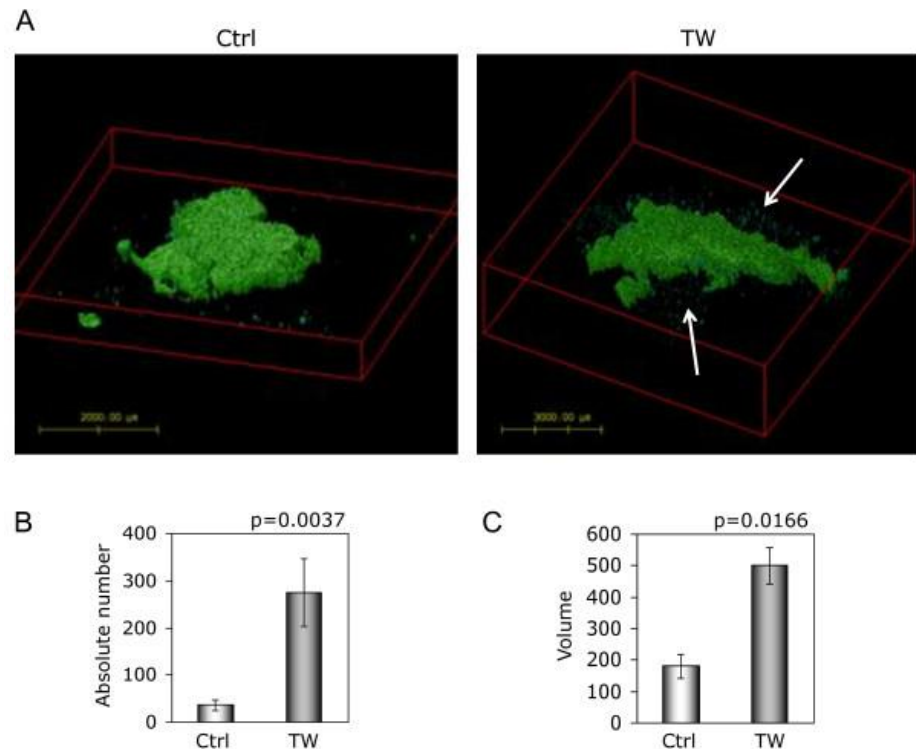


Research study currently underway:

# Theory/Hypothesis

(from what I have observed)

- **TWIST1 over-expression increased invasiveness of SNB19 cells *in vivo* (image)**
- **Hypothesis:** Knock down of TWIST1 could potentially inhibit tumor cell invasion and growth



Mikheeva *et al. Molecular Cancer* 2010 **9**:194 doi:10.1186/1476-4598-9-194

# Research study currently underway: Methods/Personal contribution

(from what I have observed)

- 4 control mice (regular BGM)
- 5 test mice (5 fold knock down of TWIST1)
- ...of the control mice: **3 died 57 days** post injection, **1 died 59 days** post injection
- ...of those with the TWIST1 knock down: **all were alive 65 days** post injection (then sacrificed)
- Created Kaplan-Meier survival graph to determine whether or not the data was statistically significant (sacrifice?)
- Assisted in the removal of the brains
- Observed brains under microscope (control was highly invasive; “TWIST1” was nearly entirely lacking tumor cells)

# Valuable experience: Thank you

- *...E-mail asked us to comment on our experience*
- Entered with open mind; search of career choice
- OR observations
- Need for immediate gratification (bacteria in incubator)
- Interest in clinical applications; ending the internship on a great note
- Grand rounds

# References

- Mikheev AM, Stoll EA, Mikheeva SA, Maxwell JP, Jankowski PP, Ray S, et al. A syngeneic glioma model to assess the impact of neural progenitor target cell age on tumor malignancy. *Aging cell*. 2009 Aug;8(4):499-501
- TWIST1 promotes invasion through mesenchymal change in human glioblastoma  
Svetlana A Mikheeva\* , Andrei M Mikheev\* , Audrey Petit , Richard Beyer , Robert G Oxford , Leila Khorasani , John-Patrick Maxwell , Carlotta A Glackin , Hiroaki Wakimoto , Inés González-Herrero , Isidro Sánchez-García , John R Silber , Philip J Horner and Robert C Rostomily  
*Molecular Cancer* 2010, 9:194doi:10.1186/1476-4598-9-194