

MY STUDENT SUMMER PROGRAM EXPERIENCE

DEPARTMENT OF NEUROLOGICAL
SURGERY:
DR. MANUEL FERREIRA LAB

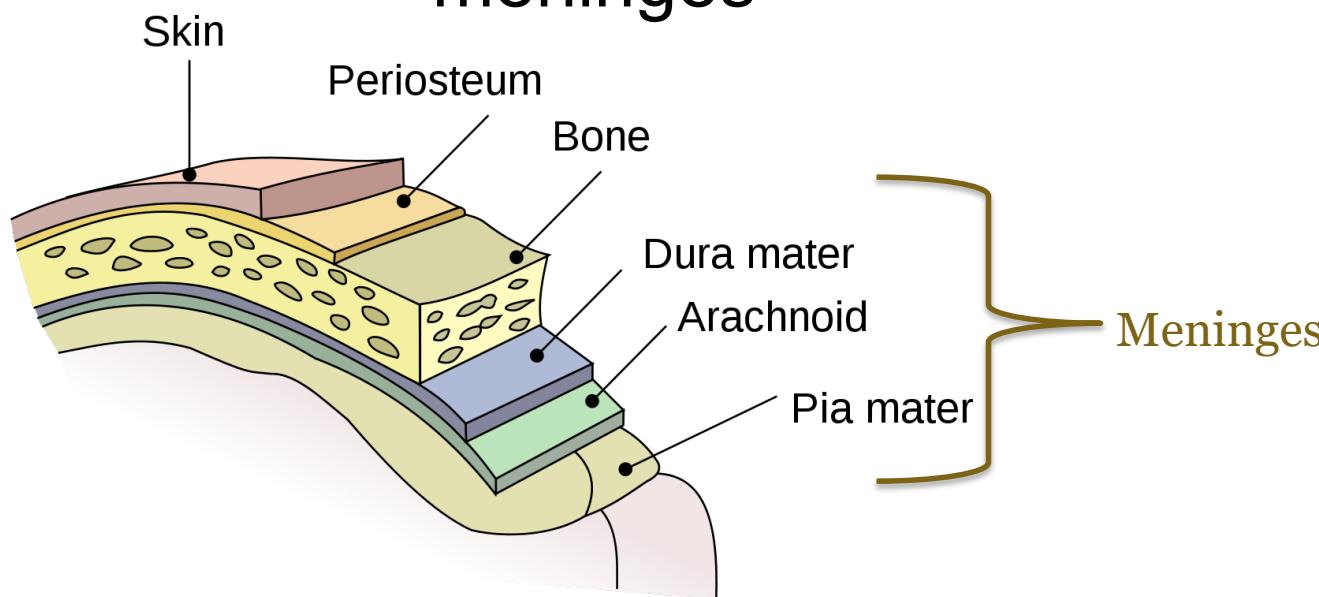
ADAM VESOLE
8/15/2014

LAB FOCUS

- Meningiomas
 - Explore various genes that could serve as biomarkers for meningioma development and metastasis
- Aneurysms
 - Beginning stages (proteomic analysis of about a dozen patients)
- Pituitary Adenomas
 - Attempting to establish viable cell line

MENINGIOMAS

- Skull-based tumors that originate from the meninges (membrane) surrounding the brain
 - Arise from the arachnoid cap cells of meninges

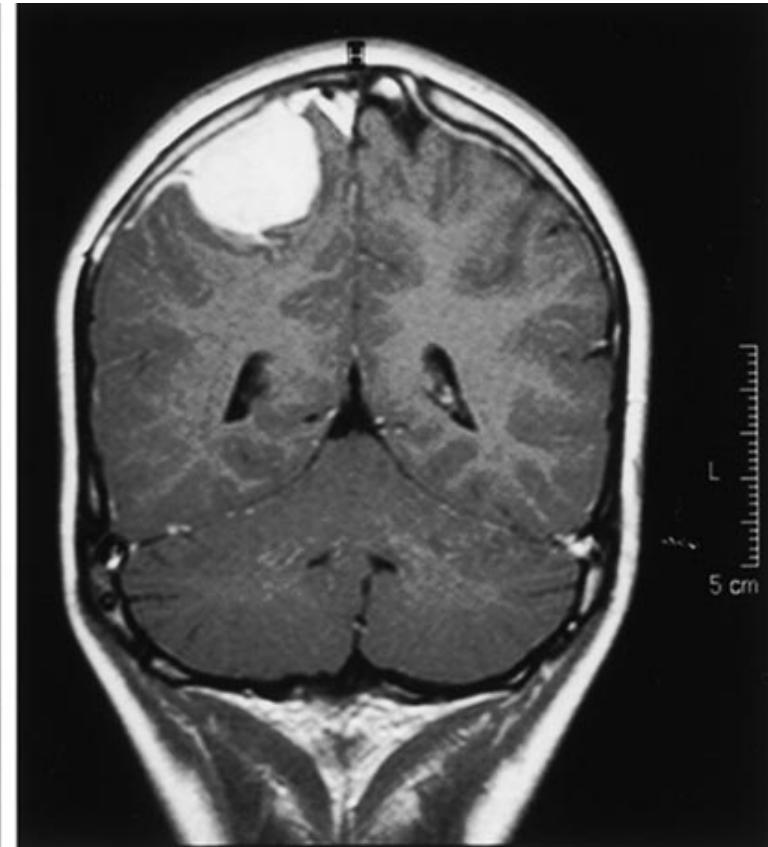
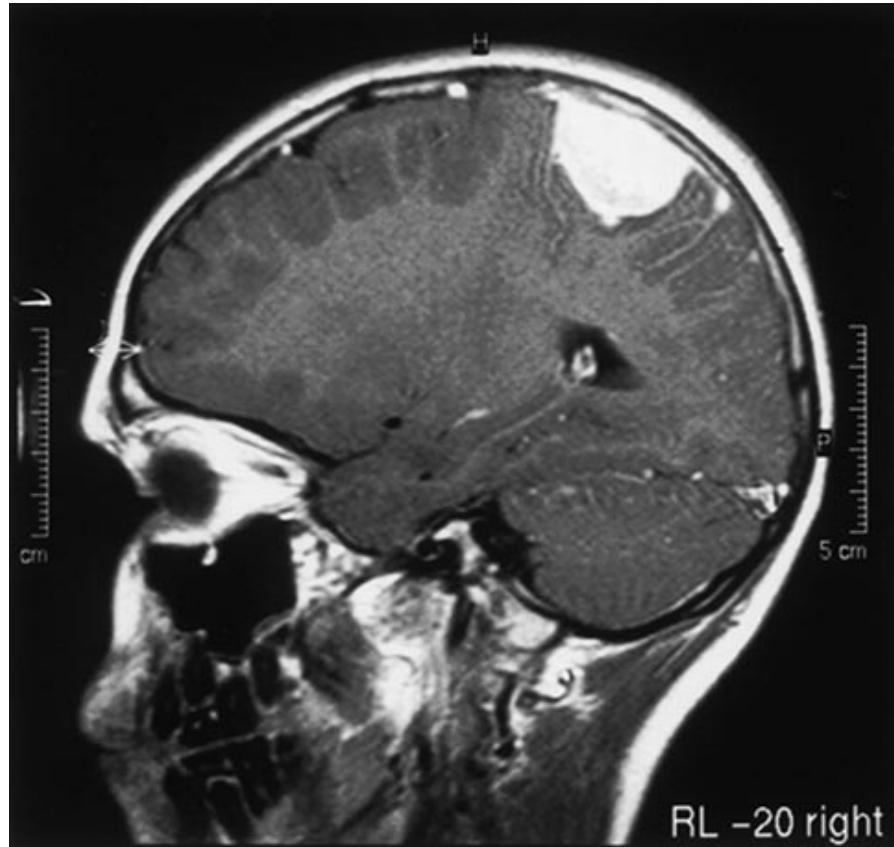


DIAGNOSIS AND TREATMENTS

- 3 Grades of Meningiomas
 - Grade I- Benign tumors (~80% of meningiomas)
 - Grade II- Atypical tumors (~15-20% of meningiomas)
 - Grade III- Anaplastic tumors (1-4% of meningiomas)
- Clinical Treatment Options
 - Observation
 - Surgical tumor resection
 - Surgery + Radiation therapy
 - Radiosurgery (gamma-knife)



MENINGIOMA MRI



FROM THE OR TO THE LAB

- For Dr. Ferreira's meningioma resections at Harborview:
 - Blood and meningioma tissue are collected and stored in tumor bank
- Weekly visits to OR to observe and collect meningioma samples from tumor bank
- Samples brought back to lab, aliquoted, and stored in freezer until needed for exome sequencing or proteomic analysis



EXOME SEQUENCING AND ANALYSIS

- Samples sent for exome sequencing
- Analyzed in lab
 - Looked for recurring gene mutations in clinical data
 - Compared our data to similar literature using Catalogue of Somatic Mutations in Cancer (COSMIC)
- Next step: Determine genes of interest and manipulate cell line to express knockdown of gene
 - Observe effects of knockdown on tumor metastasis, invasion, migration, and viability using functional assays

GENE TARGETS

Tumor	Grade	Chr22 loss	NF2	TRAF7	AKT1	KLF4	SMO
MN-95	1	Yes					
MN-290	1	Yes					
MN-1041	1	Yes					
MN-1047	1	Yes					
MN-1137	1	Yes					
MN-47	1	Yes	p.Q453X				
MN-52	1	Yes	p.F256fs				
MN-71	1	Yes	p.T59fs				
MN-81	1	Yes	p.Q65fs				
MN-169	1	Yes	p.E460X				
MN-288	1	Yes	p.K17_M29del				
MN-291	1	Yes	p.I210fs				
MN-293	1	Yes	p.Q459X				
MN-294	1	Yes	c.363+1G>C				
MN-297	1	Yes	p.K99fs				
MN-301	1	Yes	p.W41fs				
MN-306	1	Yes	p.K44X				
MN-1091	1	Yes	p.L14fs				
MN-1133	1	Yes	p.Y207fs				
MN-26	1			p.C388Y	p.E17K		
MN-105	1			p.R641C	p.E17K		
MN-292	1			p.Q637H	p.E17K		
MN-191	1			p.K615E			
MN-201	1			p.L580del			
MN-249	1			p.R641C			
MN-1025	1			p.G536S			
MN-1066	1			p.N520S			
MN-303	1			p.S561N			
MN-206	1			p.G390E			
MN-304	1			p.R653Q			
MN-305	1			p.G536S			
MN-1053	1			p.E353insFRRDAS			
MN-1045	1						p.L412F
MN-1132	1						p.W535L
MN-164	2	Yes					
MN-22	2	Yes	c.115-1G>A				
MN-54	2	Yes	p.Q319X				
MN-96	2	Yes	p.L14fs				
MN-97	2	Yes	p.M426fs				
MN-171	2	Yes	p.L208P				
MN-295	2	Yes	p.E103fs				
MN-298	2	Yes	p.V24fs				
MN-1054	2	Yes	p.R262X				
MN-16	2	Yes		p.T145M	p.E17K		
MN-1144	2	Yes		p.F337S			

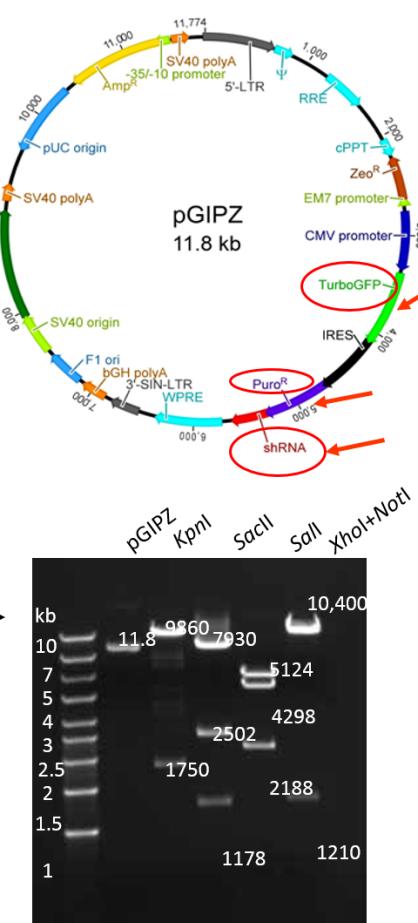
Fig.1; Clark et al., 2013: Genomic Analysis of Non-NF2 Meningiomas Reveals Mutations in TRAF7, KLF4, AKT1, and SMO

UW Medicine

- Based on literature and our clinical proteomic data of meningiomas:
 - TRAF7
 - AKT1
 - KLF4
 - SMO
 - Ptch
 - AKAP12
- Researched some of the pathways these proteins are involved in
 - Form hypothesis and begin experimentation

KNOCKDOWN OF AKAP12

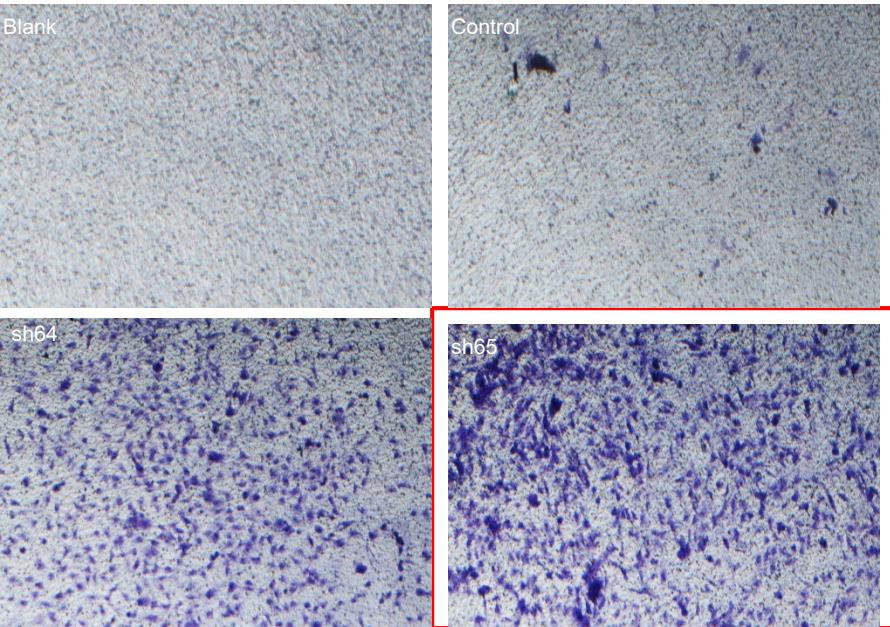
Restriction Analysis



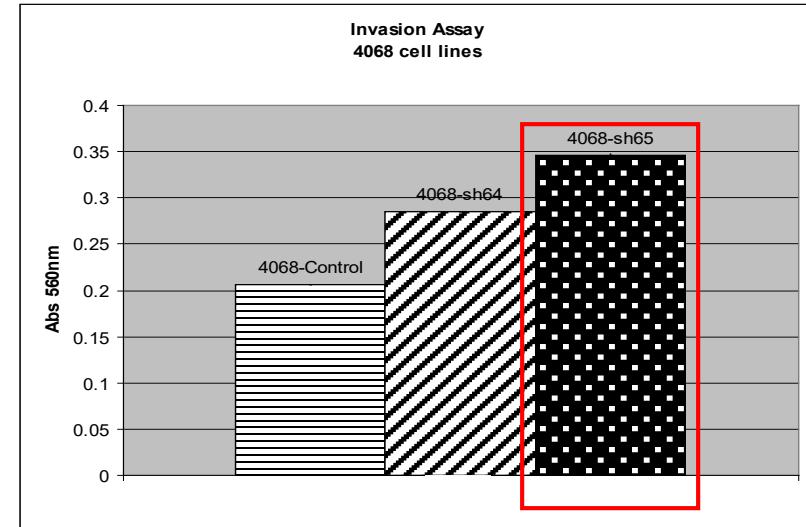
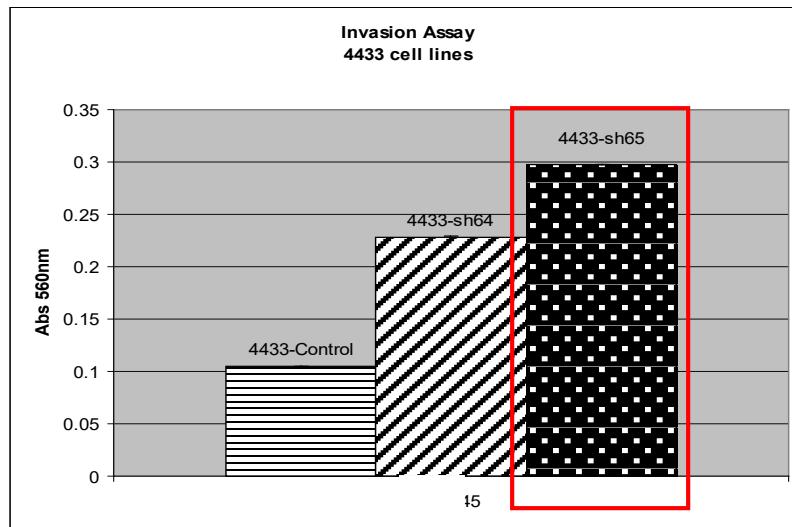
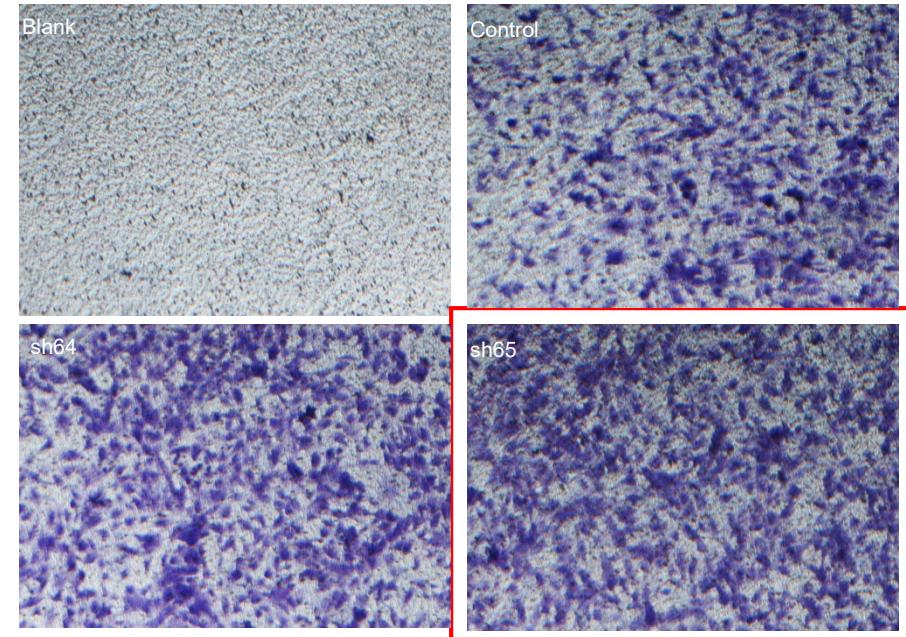
- AKAP12 was found in multiple samples and showed decreased expression in higher grades
- Transfected 2 types of meningioma cell lines
 - Inserted plasmid containing shRNA for knockdown of AKAP12

INVASION ASSAY

4433 cell lines



4068 cell lines



RESULTS SUMMARY

- Invasion and migration increased significantly with AKAP12 KD
- Proliferation assays showed no significant difference between wild type and AKAP12 KD
- Indicates that AKAP12 likely has role in regulating meningioma metastasis and unclear for proliferation



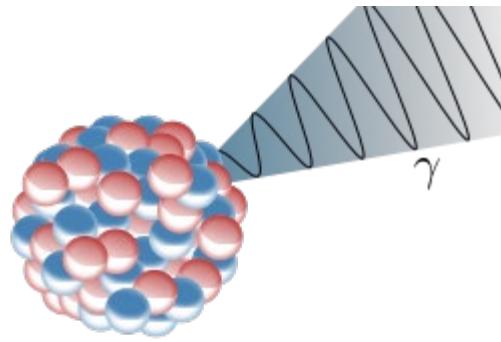
INVASION
MIGRATION



PROLIFERATION

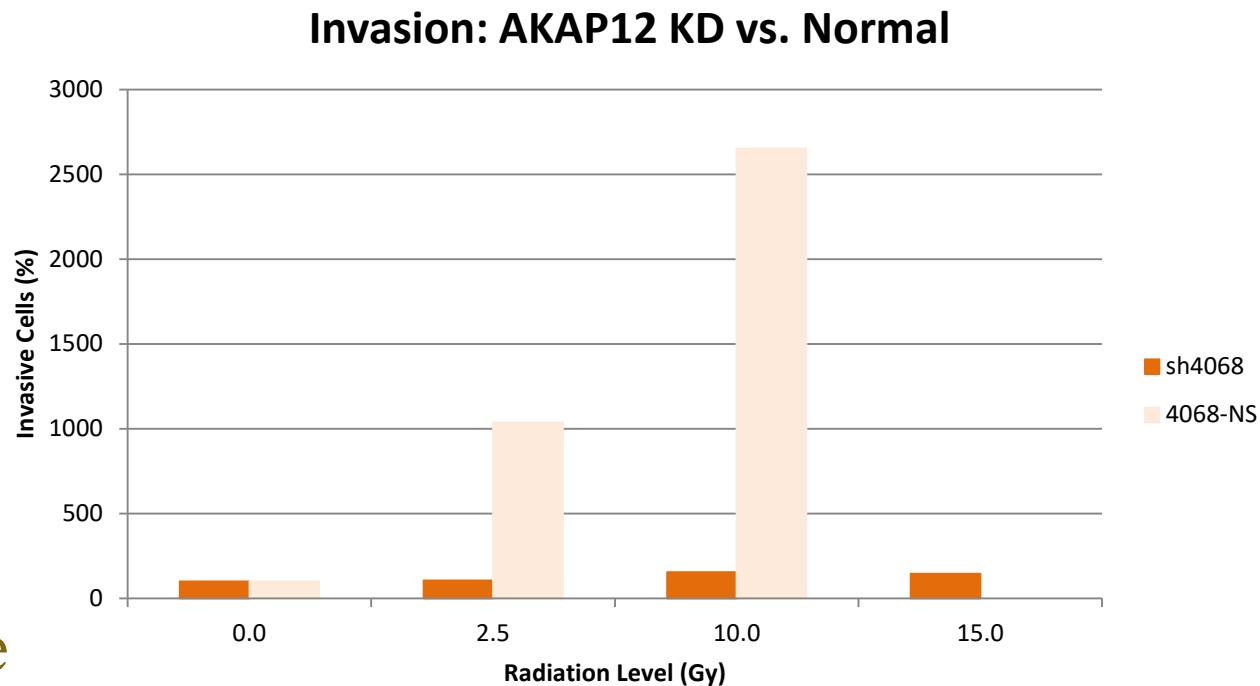
CURRENT EXPERIMENT

- Does the combination of AKAP12 loss and irradiation increase meningioma metastasis?
 - Testing the sensitivity of AKAP12 KD cells to various doses of γ -radiation



INVASION RESULTS

- Relative migration levels remained fairly constant when compared to normal meningioma cells
- Cells with low AKAP12 expression will be less sensitive to irradiation



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