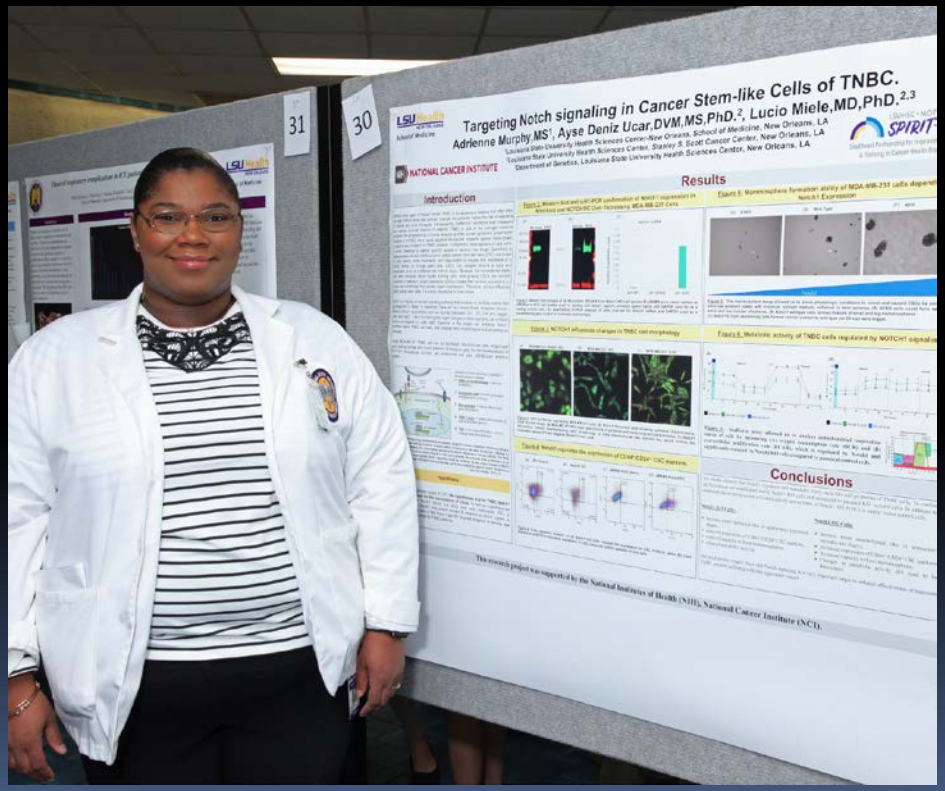
GUIDELINES: MEDICAL STUDENT VIRTUAL POSTER SESSION 2020

Dr. Fern Tsien
Assistant Dean of Medical Student Research
Department of Genetics
LSUHSC



Medical Student Research Symposia

- Dr. Paula Gregory and I started the Summer Internship Program in 2003
- Initially, poster presentations for high school and undergraduates, and oral presentations for medical students
- Since 2012, poster presentations for med students
- First virtual poster session for undergraduates at LSUHSC, July 2020

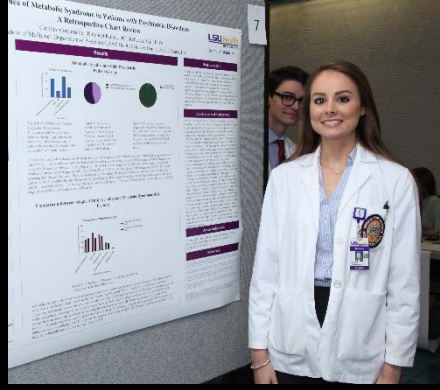
https://www.medschool.lsuhsc.edu/genetics/2020_summer_internship_poster_session.aspx

- First virtual poster session at LSUHSC for medical students, October 26-29, 2020

Important Deadline #1: Let us know you are participating

- If you plan to participate in the Poster session, please let us know by sending an email to: SoMHonorsProgram@lsuhsc.edu by the end of the day tomorrow, September 30th so we can add you to the list.
- You do not need to be an Honors Program student to participate.
- However, all Honors Program students are expected to participate.
- There were more than 100 students who did research this year, so we want to make sure you are included.
- We will send you updates and notifications, abstract and poster templates, etc.

Important Deadline #2: Abstracts



- Abstracts are due by the end of the day on Monday, October 5th
- Please send them to: SoMHonorsProgram@lsuhsc.edu using the template and guidelines that will be sent to you.
- It can be the same one from the summer if you are presenting the same project but make sure you use the template we will send you tomorrow.
- We will use the abstract you send us to generate the Abstract Book and to populate the website. This will be sent to the judges ahead of time.

Your Name (first, middle initial, last)
L1, L2, L3, or L4 (please indicate academic rank)
LSU Health Sciences Center, New Orleans, LA

Mentor's Name:
Mentor's Affiliation (LSUHSC, Department of X; Children's Hospital, etc.)

"Title of Project"

Body of Abstract: Left Justified, 11 point Arial font, single-spaced, double space between paragraphs.

Special Instructions: The abstract is a summary of the project. Do not to exceed one page. Do not change margins, font style or font sizes on this page. **Use this format only- do not modify!!!**

The Abstract

- An abstract is a one-page summary of your project.
- List your name and mentor's name as described in the template.
- Affiliations: department and school/hospital of mentor.
- Use only the template we provide.
- List academic rank: there will be L1/L2 winners and L3/L4 winners
- This template has the correct sized fonts and sizes we will use. Do not change the font or size!
- Make sure your mentor approves of your abstract before you send it to us!
- When you submit your abstract in Word format, please be sure to save the file with your last name listed first. For example: BrunoKirstenAbstract.doc
- Send it by October 5th to: SoMHonorsProgram@lsuhsc.edu

What is wrong with this abstract?

Previous studies have shown that tryptophan, melatonin, and proanthocyanidin within cherry juice may play essential roles in promoting sleep. This study utilizes cherry juice standardized to its proanthocyanidin content and tests its effectiveness as a treatment for insomnia, a common health problem in the elderly. Ten participants with insomnia complete two treatment periods (cherry juice and placebo juice), 2 weeks each, separated by a 2 week washout period. Each day the participants consume 8 ounces of juice in the morning and again 1-2 hours before bedtime. Overnight polysomnography (PSG) is used at the end of each treatment period to evaluate sleep architecture such as the distribution of sleep stages, sleep latency and state transitions. Blood samples are also taken to measure serum concentrations of free tryptophan and kynurenine in order to investigate a possible mechanism of action. Questionnaires are given before and after each two week treatment period for comparison of each treatment's effects. This study is still ongoing and data analysis will be performed upon its completion.

Nicholas Todd Alexander
Medical Student
Meharry Medical College, Nashville, Tn.

Dr. Joy Sturtevant Ph.D.
LSUHSC-SOM, Department of Microbiology, Immunology, & Parasitology

"Reprogramming innate immune system by *Shigella*: Setting the Stage"

Abstract

Shigella is an enteropathogenic bacteria that is a major cause of dysentery in developing nations and is responsible for at least 100 million cases and 1 million deaths, normally in children under the age of 5. Approximately, 500,000 cases of Shigellosis are reported annually in the United States of America and outbreaks due to multi-drug resistant strains have been reported. The course of infection is invasion of colonic epithelial cells after ingestion and causes an inflammatory diarrhea. *Shigella* is able to survive in the host because it alters host signaling pathways.

Shigella proteins have been identified which alter host signaling pathways and several of these are members of a novel E3 ubiquitin ligase family, IpaH. A major function of ubiquitin is targeting proteins for proteasomal degradation. Several IpaH proteins target the NF-kB pathway. NF-kB is a transcription factor, used to activate many genes of the innate immune system. *Shigella* is an infection of the gastrointestinal tract but IpaH targets have been identified in a human cervical epithelial cell line. Therefore, the overall goal of the lab is to identify E3 ligase cellular targets in disease relevant cells. These cell lines will be used in a novel functional screen in which the ligases will be stably expressed in an intestinal epithelial cell line, CACO-2, and differences in the resulting proteasome compared between cells harboring active versus dominant negative ligases.

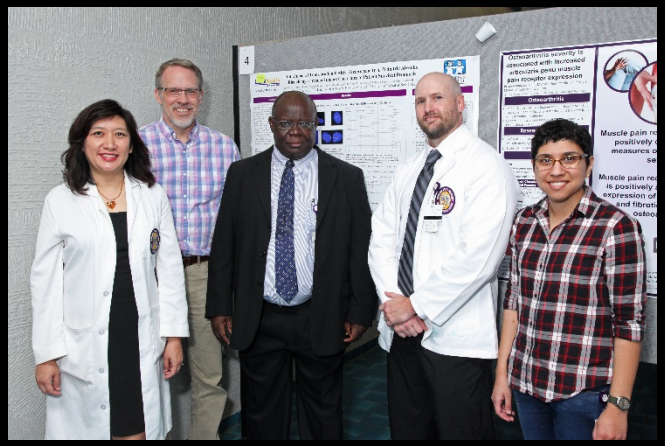
The focus of this study was to develop and optimize reagents and immunological assays in order to confirm that *Shigella* alters NF-kB signaling in an intestinal epithelial cell line- CACO-2, a physiologically relevant cell line.

We first confirmed transfection efficiency in the more malleable, embryonic epithelial kidney cell line, HEK-293A using pMAX-GFP, then analyzed NF-kB activity using luciferase reporter plasmids. We tested the NF-kB signaling pathways using several stimuli, including *Candida albicans* and *Shigella*. Once optimized, these assays will provide a platform to test the stably transfected intestinal epithelial cell lines. This data will be a reference point for the design of new therapeutic agents for *Shigella* and other enteric bacteria.

Important Deadline #3:

- Posters are due by the end of the day Friday, October 9th !

Preparing the posters



- First and most important: make sure that your mentor approves of the information that will be presented in the poster.

- Second most important: Your name should go first, your mentor's name last, and everyone else who helped you (other students, post-docs, etc.) in the middle. Make sure not to leave out anyone who helped you!

Preparing the posters, cont.

- Use the Power Point poster template sent to you by Ms. Kirsten Bruno (not your friend's or past interns) with the proper logos.
- These correspond to the funding source.
- The logos on your poster may differ from the ones on your lab mates! Do not change them!
- Use at least a 24 point font size so the text will be visible from 3 feet away.
- Feel free to adjust the box sizes and headings depending on the amount of text or figures you have.
- The poster template are already set to 34 x 44 in.

Preparing the posters, cont.

- Use any color you want to. Express yourself! Exceptions:
  - Black or deep blue for background of entire poster.
  - Image enlarged to cover the entire background.
- Spell out any acronyms the first time you use them. People outside of your lab may not know what “SIV” or “FSHD” is.
- Refer to guidelines sent to you.

Once your poster is done:

- Save it as a PPT *and* PDF file.
- When you submit your poster, be sure to save the files with your name listed first. For example: BrunoKirstenPoster.pptx
- Send the two files by Friday, October 9th, 2020 to: SoMHonorsProgram@lsuhsc.edu
- Let us know if you want us to print one for your lab

What is wrong with this poster?

PATRICK F. TAYLOR FOUNDATION

Effect of Gain-of-Function Mutant Rb on the Sphere-Forming Ability of Cell Lines

Your name goes first, Graduate students and post-docs that helped you, Mentor is last

Mentor's department and University

LSU Health Sciences Center

Abstract

Osteosarcoma, the most common bone cancer, is the second highest cause of cancer-related death in children and adolescents. Approximately 90% of cases show micro-metastasis at diagnosis, making systematic chemotherapy the first choice of treatment. Despite intensive chemotherapy, the survival rate for high-grade osteosarcomas remains at only 50-80%. This persistence is mainly due to the ability of osteosarcoma cells to metastasize and develop resistance to therapy. Increasing evidence suggests that cancer stem cells (CSCs) or tumor initiating cells (TICs) are responsible for these properties and that the inadequacy of current treatments may be result from the inability to target CSCs or TICs in osteosarcoma. Recently, we have demonstrated that small populations of osteosarcoma cells can grow and form spheres in both serum- and anchorage-independent manners. Importantly, as few as 200 cells from these spheres efficiently initiated osteosarcomas in tumor transplantation models. These results suggest that spheres are enriched with osteosarcoma CSCs. Approximately 50% of osteosarcomas contain alterations in the tumor suppressor *p53* gene. Many hotspot p53 mutants show oncogenic functions by their gain-of-function phenotypes such as increased transformation, metastasis and drug resistance, which can not be explained simply due to loss of wild-type p53 function. Our preliminary results indicate that downregulation of a gain-of-function mutant p53R172H results in a dramatic reduction of sphere-forming ability of an osteosarcoma cell line expressing p53R172H. These results suggest that mutant p53 gain-of-function is involved in sphere-forming ability and possibly CSC-like properties of osteosarcoma. However, the exact molecular mechanisms which contribute to sphere formation and CSC-like properties and the involvement of mutant p53 in these cellular phenotypes remain unclear.

Our long-term goal is to identify the molecular mechanism underlying the CSC-like properties of osteosarcoma. *The objective of this study* is to investigate the effects of several hotspot p53 mutants on the sphere-forming ability of human osteosarcoma cell lines. *Our hypothesis is that gain-of-function p53 mutants increase the sphere-forming ability of osteosarcoma cells.* To test our hypothesis, we first characterized the sphere-forming ability of several available human osteosarcoma cell lines, such as U2OS (p53 wild-type), SJSA1 (p53 wild-type), MG63 (p53-null), Saos-2 (p53-null), and KHOS (p53R156P). We found that U2OS and MG63 cell lines did not show any sphere formation when 500 cells were tested for 2 weeks of culturing in sphere-specific conditions. These results may suggest that the presence of wild-type p53 is not crucial for the sphere formation. Assays for other cell lines are on-going. We next infected MG63 cells with retroviral vectors encoding p53R175H/72P, p53R175H/72R, p53R248W/72P, p53R248W/72R, p53R273H/72P, and p53R273H/72R to establish MG63 subcell lines expressing several gain-of-function p53 mutants together with different p53 codon 72 single nucleotide polymorphisms (SNPs), since the SNP is shown to affect colony-forming ability of human cancer cell lines. Sphere formation assays using these subcell lines are underway and all results will be presented. Completion of our study will provide a better understanding of the role of gain-of-function mutant p53 in sphere-forming ability of osteosarcoma as well as useful information to dissect the molecular mechanism of CSC-like properties of osteosarcoma.

Introduction

Osteosarcoma is a devastating disease in children and young adults. In approximately 90% of osteosarcoma cases, micro-metastases are present during diagnosis, making chemotherapy the first choice of treatment. Despite intensive chemotherapy, the survival rate for high-grade osteosarcomas remains at only 50-80%. This persistence is mainly due to the ability of osteosarcoma cells to metastasize and develop resistance to therapy. Increasing evidence suggests that cancer stem cells (CSCs) or tumor initiating cells (TICs) are responsible for the metastatic and drug-resistant properties of cancer cells and that the inadequacy of current treatments for high grade osteosarcoma may result from the inability to target osteosarcoma CSCs. CSCs represent a small fraction of a tumor's cellular population and have the ability to generate new tumors identical in cellular composition to the tumor of origin. CSCs possess the abilities of anchorage-independent, serum-independent cell growth (sphere formation), tumor initiation, self-renewability, and multilineage differentiation, as well as properties of high metastatic potential and drug resistance. We have recently reported that small number of osteosarcoma cells form spheres and these spheres are enriched with cells having CSC-like properties such as high metastatic and drug resistant properties. However, the molecular mechanism that regulates CSC-like properties of osteosarcoma remains unclear.

Cancer can arise through alterations to genes that regulate cell proliferation, apoptosis, and senescence. The tumor suppressor p53, one of the key guardians of these events, exerts its functions through transactivating numerous downstream targets. Tumor suppressor p53 has a single nucleotide polymorphism (SNP) at codon 72 which is either proline (P) or arginine (R). Recent studies have shown that the 72R form is more potent in its ability to induce apoptosis compared to the 72P form. In addition to the polymorphism, mutations in the *p53* gene affect the p53 activity. Mutations in the DNA binding domain attenuate the function of p53 as a transcription factor, thereby losing its tumor suppressor activity. The importance of p53 mutation is emphasized by the clinical observation that the *p53* gene is mutated in more than 50% of tumors. Mutations in the *p53* gene are also observed in approximately 70% of patients with Li-Fraumeni syndrome (LFS), a human familial cancer-prone disease. LFS is characterized by early onset of various types of tumors, including osteosarcoma. Several missense mutations such as R175H, R248W, and R273H, are the hotspot mutations in sporadic cancer as well as the germline of LFS patients. These p53 mutants show oncogenic functions by their gain-of-function phenotypes such as increased transformation, metastasis, and drug resistance, which can not be explained simply by loss of wild-type p53 function. The molecular mechanisms underlying the gain-of-function activities and if the codon 72 SNP affects the mutant p53's gain-of-function activities remain unclear. Further, although the gain-of-function phenotypes are similar to those of CSCs, the contributions of mutant p53 to the CSC-like properties are also unknown.

Methods and Materials

Cell lines. Human osteosarcoma cell lines U2OS, SJSA1, Saos-2, MG-63, and KHOS NP were purchased from American Type Culture Collection (ATCC, Manassas, VA).

Sphere culture. Cells were counted by trypan-blue staining (Sigma Biochemicals), and live cells (five per well) were plated on a 96-well ultra-low attachment plate (Corning Inc., Corning, NY, USA) in sphere-specific media consisting of DMEM/F12, progesterone (10 nM), putrescine (50 µM), insulin (12.5 µg/ml), transferrin (12.5 µg/ml), sodium selenite (12.5 ng/ml, Sigma Biochemicals), murine EGF (10 ng/ml), and murine bFGF (10 ng/ml, Pepro Tech, Rocky Hill, NJ, USA). Cells were maintained for 10 – 14 days and fresh aliquots of EGF and bFGF were added three times a week. Sphere formation was observed daily using under a phase-contrast microscopy (Nikon Eclipse TS100).

Western blotting. MG-63 cells infected with retroviral vectors encoding control empty or a mutant p53 (R175H, R248W, or R273H) with a codon 72 polymorphism were lysed with RIPA buffer (50 mM Tris-HCl pH 7.6, 150 mM NaCl, 1 mM EDTA, 1% sodium deoxycholate, 0.1% Triton X-100, 0.1% SDS) supplemented with protease inhibitor cocktail (Roche) (1 mM phenylmethylsulfonyl fluoride (PMSF), 0.2 mM sodium orthovanadate, and 100 mM sodium fluorine). Whole cell extracts were separated by SDS-PAGE and transferred onto PVDF membranes (*GE Healthcare* Biosciences). After blocking with 5% non-fat milk in 1 x Tris-buffered saline (TBS) with 0.1% Tween-20 (TBS-T), blots were incubated with anti-human p53 (DO1, Santa Cruz) and control vinculin (Fitzgerald), followed by the incubation with secondary antibodies conjugated with horseradish peroxidase (Santa Cruz). To visualize signals, Super Signal West Dura Chemiluminescent substrates (Pierce Biotechnology) were used according to manufacture instructions. The signals were detected using a Biorad *Versa Doc* detection system (Biorad)

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Figure 1

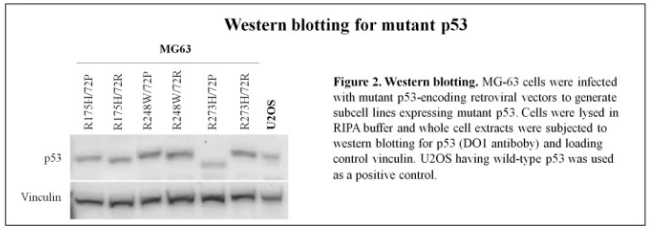


Figure 2. Western blotting. MG-63 cells were infected with mutant p53-encoding retroviral vectors to generate subcell lines expressing mutant p53. Cells were lysed in RIPA buffer and whole cell extracts were subjected to western blotting for p53 (DO1 antiboby) and loading control vinculin. U2OS having wild-type p53 was used as a positive control.

Results

Our long-term goal is to identify the molecular mechanism underlying the CSC-like properties of osteosarcoma. *The objective of this study* is to investigate the effects of several hotspot p53 mutants on the sphere-forming ability of human osteosarcoma cell lines. *Our hypothesis is that gain-of-function p53 mutants increase the sphere-forming ability of osteosarcoma cells.* To test our hypothesis, we first characterized the sphere-forming ability of several available human osteosarcoma cell lines, such as U2OS (p53 wild-type), SJSA1 (p53 wild-type), MG63 (p53-null), Saos-2 (p53-null), and KHOS (p53R156P). We found that U2OS and MG63 cell lines did not show any sphere formation when 500 cells were tested for 2 weeks of culturing in sphere-specific conditions. These results may suggest that the presence of wild-type p53 is not crucial for the sphere formation. Assays for other cell lines are on-going. We next infected MG63 cells with retroviral vectors encoding p53R175H/72P, p53R175H/72R, p53R248W/72P, p53R248W/72R, p53R273H/72P, and p53R273H/72R to establish MG63 subcell lines expressing several gain-of-function p53 mutants together with different p53 codon 72 single nucleotide polymorphisms (SNPs), since the SNP is shown to affect colony-forming ability of human cancer cell lines. Sphere formation assays using these subcell lines are underway and all results will be presented. Completion of our study will provide a better understanding of the role of gain-of-function mutant p53 in sphere-forming ability of osteosarcoma as well as useful information to dissect the molecular mechanism of CSC-like properties of osteosarcoma.

Table 1

Table 1. Results of sphere formation assays

Cell lines	p53 status	Cell# examined	# of spheres formed	% sphere formation
U2OS	wild-type	480	0	0
SJSA1	wild-type	480	1	0
Saos-2	null	480	318	66.3
MG63	null	480	0	0
MG63 R175H/72P	R175H/72P	480	84	17.5
MG63 R175H/72R	R175H/72R	480	160	33.3
MG63 R248W/72P	R248W/72P	480	217	45.2
MG63 R248W/72R	R248W/72R	480	144	30.0
MG63 R273H/72P	R273H/72P	480	112	23.3
MG63 R273H/72R	R273H/72R	480	136	28.3
KHOS	R156P	480	112	23.3

Conclusions

Conclusions

1. Spheres vary in size and rate of growth in different osteosarcoma cell lines.
2. The presence or absence of wild-type p53 does not have any effects on the sphere-forming ability of osteosarcoma cell lines.
3. The presence of mutant p53 does enhance the sphere formation of osteosarcoma cells.
4. The effects of p53 codon 72 polymorphisms vary in different p53 mutations.
5. All p53 mutants confer osteosarcoma cells with sphere-forming abilities.

Future directions

1. Examine the effects of mutant p53 on other CSC-like properties such as tumor initiating ability, self-renewal, metastatic potential, and drug resistance.
2. Examine the effects of mutant p53 down-modulation in various osteosarcoma cell lines carrying mutant p53.
3. Identify genes that regulate sphere-forming ability and CSC-like properties of osteosarcoma cells.

Example of a better poster

RNA Binding ability of FUS mediates toxicity in a *Drosophila* model of ALS

Senthil S. Natarajan, J. Gavin Daigle, Nicholas A. Lanson, Jr., John Monaghan, Ian Casci, Udai B. Pandey

Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA

LSU Health New Orleans Health Sciences Center

LVC Louisiana Vaccine Center

Abstract

Amyotrophic Lateral Sclerosis (ALS) is a late-onset neurodegenerative disorder characterized by the loss of motor neurons. Mutations in Fused-in-Sarcoma (FUS) have been identified as a major component in both familial (FALS) and sporadic (SALS) ALS cases. FUS is an RNA-binding protein implicated in several processes like RNA splicing and microRNA processing. In normal individuals, the FUS gene is predominantly localized in the nucleus; however in ALS patients, FUS becomes redistributed to the cytoplasm as well, which is believed to be a causative pathway for ALS.

Ectopic expression of human FUS with ALS-linked mutations in fly eyes causes moderate to severe external eye degeneration. Here we examined the role of RNA binding in mediating the neurodegenerative effects of mutant FUS via the RNA Recognition Motif (RRM). The RRM domain in FUS is key to the RNA binding pathway and can be disrupted by total deletion of the domain itself (RRM-D) or by mutating 4 conserved phenylalanine residues within the FUS RRM to leucine (known as 4F-L). The 4F-L mutations have been previously shown to mitigate RNA binding ability in a yeast model of FUS.

We demonstrate that disrupting the RRM-Domain, by way of deletion or by the 4F-L point mutations, can suppress the toxicity of FUS. Interestingly, confocal imaging has shown that disrupting the RNA binding-ability keeps FUS within the nucleus (unlike in ALS cases, where FUS is redistributed to the cytoplasm), further indicating that subcellular mislocalization of FUS is a causative pathway for ALS.

In summary, we have identified a means of rescuing phenotype in our *Drosophila* model of ALS-associated neurodegeneration, which may be relevant for future clinical studies and interventions in ALS.

Introduction

- Familial (genetic) ALS accounts for ~10% of all ALS cases, with mutations in FUS accounting for ~4-5% of FALS cases.
- Victims of ALS display loss of muscle mass, increased frailty, loss of mobility, and eventually death.
- Currently ALS has no definitive treatment in addition to being ultimately fatal, making the study of ALS all the more urgent and important.
- Steve Gleason, former New Orleans Saint and known ALS patient, in a simply a few years, has gone from inciting the loudest recorded noise in the Superdome with his blocked punt all the way to a man confined to a wheelchair and deprived of his former stature.

- Knowing that FUS in itself is an RNA-binding protein, we hypothesized that disruption of its RNA binding ability by deletion of the RRM domain or by 4F-L mutations would reduce the toxicity of mutant FUS.
- We started by transfecting neuronal cells with FUS and corresponding FUS mutations. We then tested our hypothesis by creating transgenic lines with a deletion of the RRM domain in FUS entirely (RRM-D). We next narrowed our focus and created transgenic lines in which we mutated 4 conserved phenylalanine residues within the FUS RRM to leucine (known as 4F-L). Both the RRM-D and 4F-L lines were used in screens in which the FUS trans-gene was expressed in the fly eyes.

I. FUS Gene Model

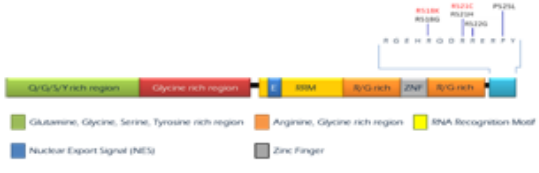


Figure 1: In 2009, ALS-causing mutations in the FUS gene were identified and led to a line of thinking that perhaps errors in RNA metabolism could be involved in ALS pathogenesis.

II. A *Drosophila* model of FUS

Lanson N A et al.

- Recently, our lab developed a *Drosophila melanogaster* (fruit fly) model as a highly useful system for studying FUS-induced proteinopathies such as ALS.
- Fly models of FUS recapitulate several key features of ALS, demonstrating pupal lethality and larval locomotion defects.

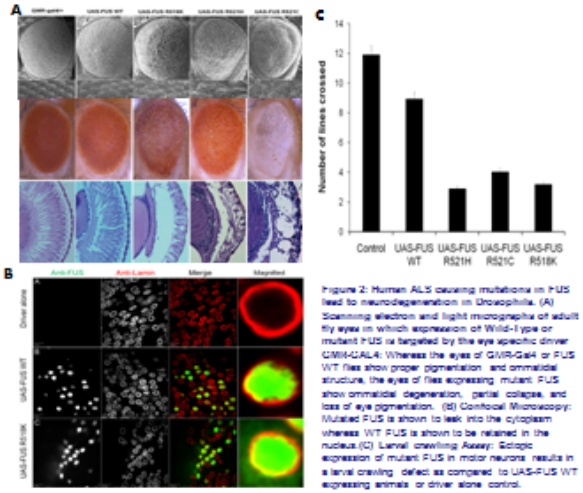


Figure 2: Human ALS causing mutations in FUS lead to neurodegeneration in Drosophila. (A) Scanning electron and light micrographs of adult fly eyes in which expression of Wild-type or mutant FUS is targeted by the eye specific driver GMR-GAL4: Whereas the eyes of GMR-Gal4 or FUS WT flies show proper pigmentation and ommatidial structure, the eyes of flies expressing mutant FUS show ommatidial degeneration, partial collapse, and loss of eye pigmentation. (B) Confocal Microscopy: Mutated FUS is shown to leak into the cytoplasm whereas WT FUS is shown to be retained in the nucleus.(C) Larval crawling Assay: Ectopic expression of mutant FUS in motor neurons results in a larval crawling defect as compared to UAS-FUS WT expressing animals or driver alone control.

III. RNA Binding ability is essential for FUS-related neurodegeneration.

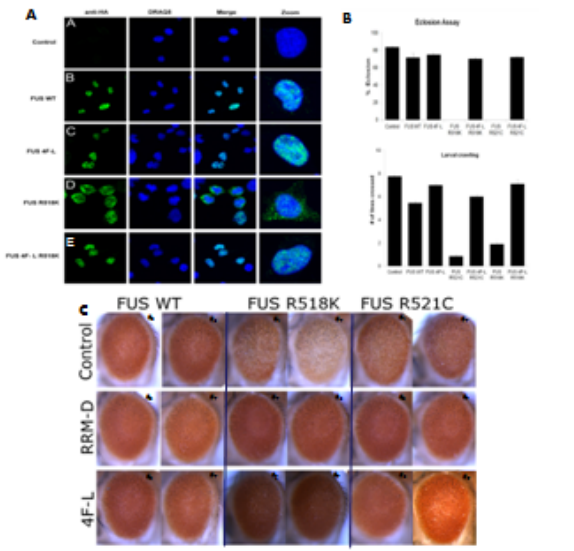


Figure 3: RNA-binding ability of FUS regulates toxicity and subcellular localization. (A) Confocal Imaging: In neuronal cells, WT FUS (B) is predominantly nuclear whereas FUS with ALS-linked mutation (D) is redistributed into the cytoplasm. RNA-binding incompetent FUS along with an ALS-linked mutation (E) is localized in the nucleus. (B) Behavioral Assays: When FUS was targeted by the motor-neuron specific driver (OK-gal4), we observed greater lethality among pupae with an ALS-linked mutation as opposed to normal eclosion in WT or RNA-binding deficient FUS. Similarly, we observed that expression of mutant FUS in motor neurons results in a larval crawling defect as compared to normal locomotion from FUS WT and non-transgenic controls. Interestingly RNA-binding incompetent larvae also displayed normal locomotion. (C) Light Micrographs of Crossed transgenic Fly lines: Expressing R518K or R521C mutations in fly eyes led to external eye degeneration. However, blocking RNA binding by deleting the RRM domain or by 4F-L mutation rescues the degenerative phenotype.

Conclusions

- Disrupting the RRM-Domain by way of deletion or by 4F-L mutations does indeed seem to significantly rescue phenotype in mutated FUS fly eyes.
- For further research, we want to express RNA-binding deficient FUS mutations in motor neurons of flies and assess neurodegeneration with respect to motility and larval crawling ability.
- We would also like to further investigate the link between subcellular localization of FUS and its toxicity, a point of interest which showed up in these experiments.

- Lanson, N. A., et al. 2011. A *Drosophila* model of FUS-related neurodegeneration reveals genetic interaction between FUS and TDP-43. Human Molecular Genetics. 20, 2510-2523.
- Lanson, N.A., Pandey, U.B., FUS related proteinopathies: Lessons from animal models, Brain Res. (2012), doi:10.1016.2012.01.039

- Acknowledgements: This work was supported by the ALS association and the Robert Packard Center for ALS at Johns Hopkins Medical Center. We would also like to thank the Louisiana Vaccine Center and LSU Health Sciences Center for their generous support.

Example of a better poster

LOUISIANA GENE THERAPY RESEARCH CONSORTIUM

AXIN2 Gene Instability In Colon Cancer

Summer Student (you), People who helped you, mentor

Mentor's department and University

LSU Health Sciences Center

Abstract

Colon cancer is one of the most prevalent and fatal cancers in the world. In the United States, 10% of all cancer patients have colon cancer. The disease begins when adenomatous polyps, fleshy growths that line up on the inside of the colon, become cancerous. Colonoscopy is often performed to detect these polyps. Regular testing after the age of 40 can drastically reduce the risk of developing colon cancer.

The AXIN2 gene, located in the region of 17q23-q25, is a gene of interest due to its interaction with the Adenomatous polyposis coli (APC) gene in the Wnt signaling pathway and its association with colon cancer with defective mismatch repair. Mutations in the Adenomatous polyposis coli (APC) gene have been found in about 85% of colon cancer patients. However, not much is currently know about the role of AXIN2 in colon cancer development. By conducting research on AXIN2, researchers are hoping that this gene may assist in distinguishing different subgroups of colon cancer. For this project, we analyzed two colon cancer cells lines to determine their karyotypic differences and for any 17q23-q25 region abnormalities.

The majority of the metaphase cells from both of the colon cancer cell lines analyzed were aneuploid, with one cell line (SW480) having a dramatically higher number of chromosomes reaching hypertetraploidy (103 chromosomes). In addition, the SW480 cell line contained some metaphase cells with an extra copy of chromosome 17 with amplification of the 17q23-25 region. This is the gene location of AXIN2, indicating the possibility of AXIN2 over-expression leading to the colon cancer in this cell line.

Introduction

The colon is the last portion of the large intestine, which also includes the rectum. Colorectal cancer (CRC), also known as colon cancer, is the third most common cancer in the world and the second most fatal cancer in the Western hemisphere. It is reported that approximately 655,000 people worldwide die from this disease every year. It usually arises from adenomatous polyps that line the inside of the colon. Mutations in certain genes are have been associated with this disease.

One significant gene known to cause CRC is the adenomatous polyposis coli gene (APC). The APC gene is located on the chromosome 5 between positions 21 and 22. Its normal function is to provide instructions for the creation of the APC protein, which helps control how and when a cell should divide. Mutations in this tumor suppressor gene can cause CRC, gastric (stomach) cancer, and Turcot syndrome. Approximately 85% of the people who have colon cancer have a mutation in the APC gene. If a person inherits just one defective copy of the gene from one of their parents, then he or she is almost guaranteed that they will develop colon cancer by the age of 40.

A gene that the APC interacts with is the relatively unknown AXIN2 gene, the focus of this project. Located on chromosome 17 between positions 23 and 24, this gene's protein, Axin2, is presumably very important in the regulation of beta-catenin, which is also a function of the APC gene. Since the APC gene and AXIN2 gene interact in the same pathway, it is believed that a mutation to either gene can affect the other gene. About 30% of the people with colon cancer with defective mismatch repair (the mechanism to correct DNA replication errors) have a mutated AXIN2 gene. The region containing the gene shows loss of heterozygosity in breast cancer, neuroblastoma, and other cancers and tumors. Deletions or mutations in this gene can result in truncated proteins which are most likely inactive. There is a possibility that somatic inactivating mutations in AXIN2 can deregulate beta catenin, and therefore, AXIN2 may be tumor suppressor gene.

Colon Cancer Symptoms

- Constipation
- Vomiting
- Stomach cramps
- Thin stool
- Diarrhea
- Hematochezia (Blood in stool)
- Unexplained Weight loss

Figure 1

The AXIN2 gene is located on Chromosome 17 on the q arm (long arm) between positions 23 and 24. The gene spans about 35 kbp and 843 amino acids.

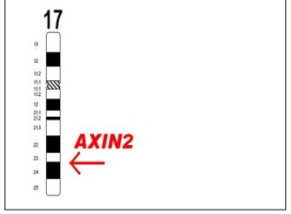
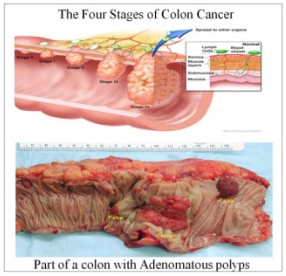


Figure 2



Part of a colon with Adenomatous polyps

Figure 3

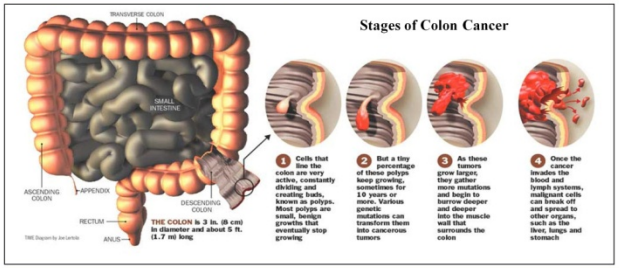
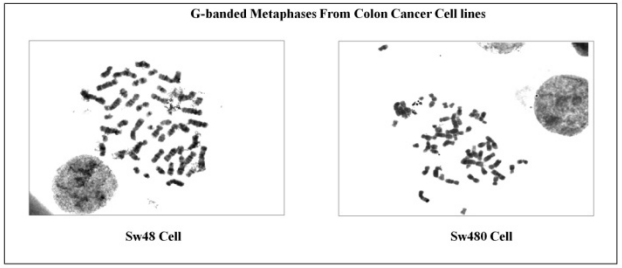


Figure 4



Methods and Materials

Samples and Culture Conditions:

Two colon cancer lines were obtained from human patients. The Sw48 cell line was obtained from an 82 year old female and the SW480 cell line was obtained from a 50 year old male. The cells were grown in DMEM with 10% Fetal Bovine Serum (FBS) and 1% penicillin under normal culturing conditions.

Chromosome Preparation:

For solid staining and G-banding, cells were harvested in exponential phase, incubated with colcemid, treated with a KCL hypotonic, and fixed two times with methanol and acetic acid. For solid staining, the cells were dropped onto slides and stained with Giemsa. For G-banding, the cells were dropped onto slides, followed by a short incubation in a trypsin solution prior to staining with Giemsa.

Results

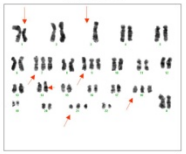
The table to the right shows the frequency of different ploidies in the Sw48 and Sw480 colon cancer cell line.

Ploidy of Human Colon Cancer Cell Lines

	Sw48	Sw480
Total # of cells analyzed	35	20
Diploidy = 46 (Normal #) (%)	2 (6%)	0 (0%)
Hyperdiploidy 47-57 (%)	33 (94%)	6 (30%)
Hypotriploidy 58-68 (%)	0 (0%)	8 (40%)
Triploidy = 69 (%)	0 (0%)	0 (0%)
Hypertriploidy 70-80 (%)	0 (0%)	1 (5%)
Hypotetraploidy 81-91 (%)	0 (0%)	4 (20%)
Tetraploidy 92 (%)	0 (0%)	0 (0%)
Hypertetraploidy 93-103 (%)	0 (0%)	1 (5%)

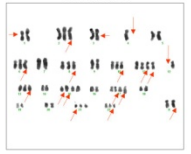
G-Banded Karyotypes Representative of Colon Cancer Cell lines. The Red Arrows indicate abnormalities.

Sw48 Cell



49, XX, Del (1), (p31), -3, +7, +9, inv (14) (q11q22), +18, +21

Sw480 Cell



57, X-Y, +der X, iso (1q), +2, iso (3q), -4,+6, +8, +10,+11,+11, -12, +13, +15, +17, +add (17) (q23), +21, +22

Conclusions and Future Directions

When compared to normal human diploid cells, the majority of the cells from the Sw48 cell line were hyperdiploids ranging from a total of 47 to 57 chromosomes per cell, while the Sw480 cell line had a wide range of total chromosome number ranging from hyperdiploidy to hypertetraploidy (up to 103 chromosomes). Our results had many similarities with published literature on these cell lines. For example, both previously published and our analysis of sw40 showed the presence of some diploid cells as well as some hyperdiploidy, with an extra chromosome 7 in common.

The sw480 cell line was much more unstable in both studies, with common abnormalities including a missing Y, an extra X abnormal X chromosome, isochromosome 3q, and trisomy 13, 21, and 22. The previous report found one extra chromosome 17. However, our results show four 17 chromosomes, with one of them containing additional genetic material at the q23-qter, the critical region of the AXIN2 gene. Fluorescence *in situ* hybridization (FISH), RNA, and protein analyses should be preformed to determine the extent of AXIN2 amplification in the Sw480 cell line.

Due to the nature of these immortalized cell lines, chromosome abnormalities are acquired with increased cell proliferation. *In vitro* studies such as this one can help to gives an idea of what can occur *in vivo*. More cancer cell lines should be analyzed in order to find genetic differences between the various types of colon cancer.

Geriatric Depression Scale Scores Correlate With Changes in the Oral Microbiota and Abundances of Opportunistic Pathogens in HIV Positive Individuals

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Introduction

Several studies provide evidence that there is a link between depression/mental illness and microbial communities, particularly the gut. However, there has been little research into the link between depression and the population of oral microbiota, especially for individuals with Human Immunodeficiency Virus (HIV).

We hypothesized that there will be a significant difference in the oral microbiota of individuals with depression and those without depression. Furthermore, we hypothesized that HIV positive patients with depression will show a higher abundance of opportunistic pathogens than patients without depression. Similarly, the abundance is expected to be higher for HIV negative patients with depression when compared to those without depression.

Sample Demographic

	0-5	6-15
n	51	25
Male	38 (74.5%)	10 (40%)
Female	13 (25.5%)	15 (60%)
HIV (+)	37 (72.5%)	20 (80%)
HIV (-)	14 (27.5%)	5 (20%)
Age (yr)	50.4 ± 5.5	50.6 ± 7.3
White	19 (37.3%)	6 (24%)
Black	33 (64.7%)	19 (76%)
American Indian	1 (2%)	0 (0%)
Smoking	36 (70.6%)	17 (68%)
Alcohol	51 (100%)	23 (%)
Drug Abuse	32 (62.7%)	20 (80%)

Table 1. Samples were grouped into the categories of those with a GDS score of 0-5, and a GDS score of 6-15. The subgroups (Sex, Race, Female, etc.) are reported as the number of samples that meet the subgroup criteria followed by the percentage.

Alpha Diversity

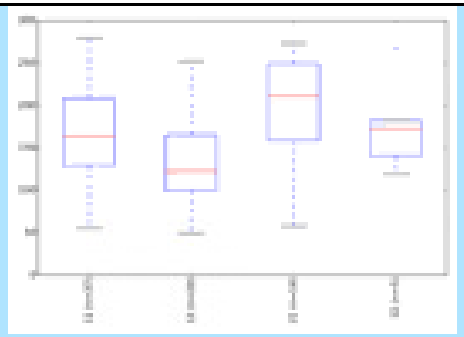


Figure 1. Chao1 Box Plot. The box "between" of the groups, HIV+ (GDS 6-15) and HIV- (GDS 0-5), were the only two groups compared to show any statistical significance ($p = .05$). We expected these groups to differ significantly. However, we did not expect the other comparisons to come back as not statistically significant.

Unifrac Pcoa Plot

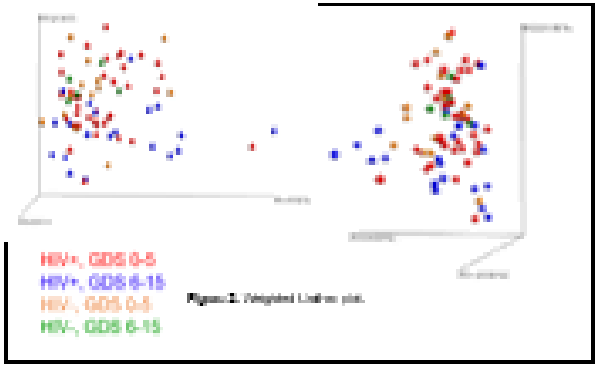


Figure 2. Weighted Unifrac plot.

Taxonomic Summary

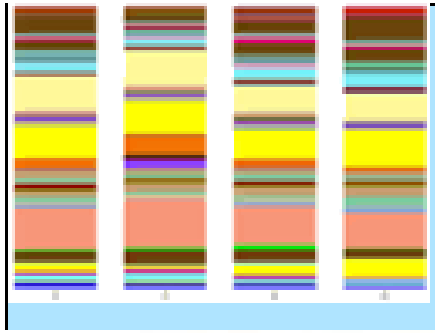


Figure 3. Samples were categorized based on HIV Status, and GDS, Geriatric Depression Scale, score. The x-axis represent these categories. The first number indicates HIV status, a one is positive, two is negative. The second number represents GDS ranking, a one represents those with a GDS score of 0-5, and a two represents a GDS score of 6-15. Only the top 20 pathogens explored showed up above 1% overall abundance. They are listed below with their percentages in each group.

Taxonomy	Total	HIV+, GDS 0-5 (37)	HIV+, GDS 6-15 (20)	HIV-, GDS 0-5 (14)	HIV-, GDS 6-15 (5)
Prevotella Intermedia	0.8%	0.8%	0.9%	1.7%	0.3%
Prevotella Nigrescens	0.8%	0.8%	1.0%	0.7%	0.3%
Prevotella Pallens	1.0%	1.0%	1.0%	1.0%	1.0%
Streptococcus sp.	3.6%	3.6%	4.6%	2.7%	1.5%

Table 2. Out of the 20 opportunistic pathogens standard only 4 were present at higher than 1% total abundance (abundance across all groups). The table is divided into depression status by color. The gold color indicates a GDS score of 0-5, the green indicates a GDS score of 6-15. We hypothesized that those with depression would have a higher abundance of these opportunistic pathogens. This is true for 3 of the 4 in the HIV+ groups. However, this is not true for the HIV- groups, they are in closely same in groups with a leading 3.

Conclusions

- Beta Diversity didn't show an association with GDS scores.
- The majority of subjects with higher GDS scores were HIV positive
- Of the 20 pathogens investigated, only Streptococcus sp., Prevotella Intermedia, and Prevotella Nigrescens demonstrated a relationship with GDS scores and exclusively in the HIV+ groups.
- Alpha Diversity only showed statistical significance for the groups HIV+ (GDS 6-15), and HIV- (GDS 0-5).

This research project was supported by a grant from the US Dept. of Commerce held in conjunction with the New Orleans BioInnovation Center

"Unexpected Results from Hereditary Cancer Panel Genetic Testing: Do Duplications of MMR Genes Matter?"

Sophia Turner[1], Alix D'Angelo, MGC, CGC[1,2].

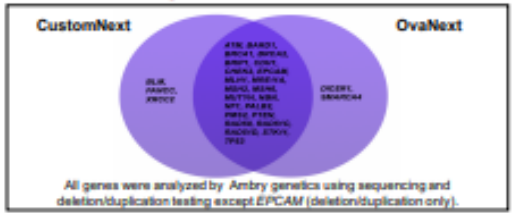
[1]Louisiana State University Health Sciences Center, Department of Genetics

[2]University Medical Center New Orleans, Cancer Center.

Introduction

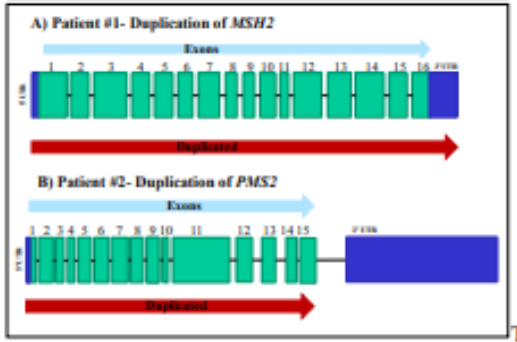
- Lynch syndrome (LS), is the most common form of hereditary colorectal cancer (up to 82% lifetime risk), and also increases the lifetime risk of a variety of cancers, including endometrial (up to 60%), ovarian (up to 24%), gastric, small bowel, hepatobiliary tract, pancreatic, urinary tract, brain and skin neoplasms.[1-3]
- LS is inherited in an autosomal dominant pattern and caused by heterozygous germline mutations in one of five genes: *MLH1, MSH2, MSH6, PMS2,* and *EPCAM*. [4]
- *MLH1, MSH2, MSH6* and *PMS2* are known as mismatch repair (MMR) genes, which play a major role in DNA repair due to replication errors.[5] *EPCAM* is not an MMR gene, however, it impacts the expression of *MSH2*.[6]
- Identifying individuals with LS is crucial, because increased surveillance and preventative surgical options are available.[7]
- We present two patients who were referred to the Genetic Counseling clinic at University Medical Center. Interestingly, both patients met *BRCA* 1/2 genetic testing criteria but were found to have a duplication of an MMR gene.
- A literature search was performed to determine whether these duplications may be of clinical significance, and therefore impact patient management.

Hereditary Cancer Panels

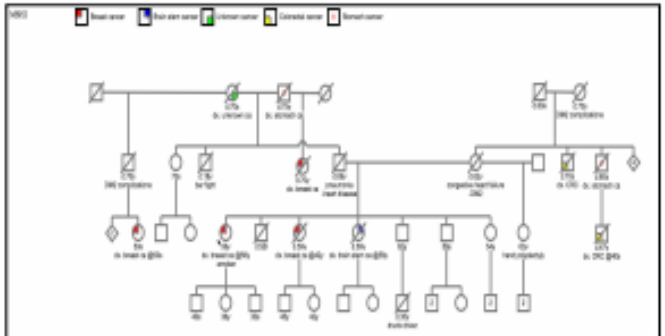


All genes were analyzed by Ambry genetics using sequencing and deletion/duplication testing except *EPCAM* (deletion/duplication only).

Genetic Test Results

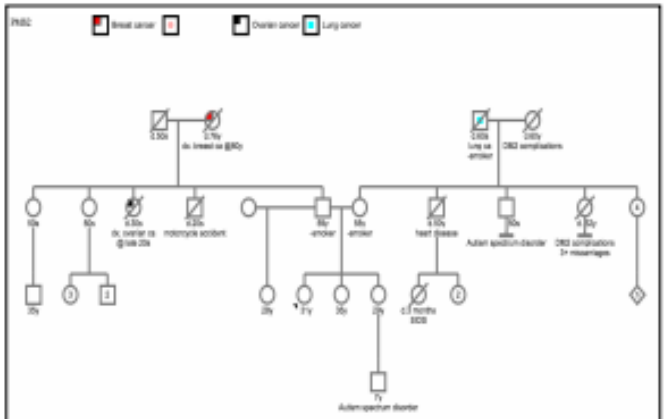


Patient #1



Patient #1 is a 57 year-old post-menopausal female referred to Genetics clinic due to her personal and family history of cancer. She was diagnosed with stage IIB ER/PR+ HER2- invasive ductal carcinoma of the left breast at 56 years-old. She underwent left mastectomy and 6 weeks of adjuvant radiation therapy. She has a history of smoking tobacco (1/2 pack per day since she was 20 years-old). Details of the family history are available in the pedigree above.

Patient #2



Patient #2 is a 31 year-old pre-menopausal, nulliparous, unaffected female referred to Genetics due to her family history of cancer. Details of the family history are available in the pedigree above.

Variant Classification Scheme

- **Pathogenic Mutation**- known to cause a disorder
- **Likely Pathogenic Variant**-strong evidence suggesting it causes a disorder
- **Variant of Unknown Significance** – limited or conflicting data
- **Likely Benign Variant**-strong evidence suggesting it does not cause a disorder
- **Benign**- does not cause a disorder

Discussion

- Next-generation sequencing technology has drastically transformed the genetic testing paradigm, particularly in the hereditary cancer specialty.[8-9] However, this testing has also led to an increase in inconclusive and unexpected results.[10]
- Despite the patients' histories of breast +/- ovarian cancer, both were found to have a whole gene duplication of an MMR gene (*MSH2, PMS2*). The families presented in this report do not meet Amsterdam II criteria, however, they are suspicious of hereditary forms of cancer considering the types of cancers, ages at diagnosis and number of affected relatives in the families.
- A recent study of 528 individuals who have a mutation in one of the MMR genes shows an interesting correlation with breast cancer. Among these individuals, 23.5% had breast cancer (compared to 35.2% who had colorectal cancer and 25.8% who had endometrial cancer), noting that breast cancer was nearly as prevalent. Additionally, individuals who had mutations in *PMS2* or *MSH6* were more likely to meet NCCN guidelines for *BRCA1/2* testing (not Lynch syndrome) than *MLH1* and *MSH2* carriers.[11] This study suggests that the presentation of our patients may be part of the LS phenotypic spectrum.
- Many different types of mutations in the MMR genes are known to be pathogenic, including missense, nonsense, deletions and partial duplications.[12-13] For example, in a report of two individuals with personal and family histories of early- and late-onset colorectal, endometrial and other cancers, exons 7-14 of *MSH2* were duplicated. While the families did not meet Amsterdam II criteria, several tumors were confirmed to have high microsatellite instability, which combined with the presentation of these patients confirmed that the duplication was responsible.[12]
- Unfortunately, evidence of whether whole MMR gene duplications are pathogenic is limited, and they are currently classified as variants of unknown significance (VUS). Pathogenic whole gene duplications have been observed in another gene that is associated with hereditary colorectal cancer/polyposis, *GREM1*.[14-15] However, GREM1 and MMR protein products serve very different functions. Additionally, there are no families that meet Amsterdam II criteria with whole MMR gene duplications that have been reported in literature, to our knowledge.
- Follow-up testing, including chromosomal microarray may be beneficial for our patients in order to further evaluate the size and location of the duplications. Further family and molecular studies are necessary to reclassify these variants, as this may have a dramatic impact on the management of patients and their families.

This research was supported by the Entergy Workforce Training Grant.

Influenza Vaccination Program Requirements of Healthcare Personnel in Louisiana Hospitals

names

LSUHSC-NOLA, Department of Pediatrics, Division of Infectious Diseases and Children's Hospital, New Orleans

Introduction

- Influenza virus causes 24,000 annual deaths in the U.S. Every year 450,000 to 900,000 Louisiana residents are infected and 800 die.
- To prevent high morbidity and mortality, annual vaccination of patients and healthcare personnel (HCP) is recommended. Yet, the vaccination coverage of U.S. HCP in 2010 was only 60%.
- In response, the Centers for Disease Control and Prevention (CDC) is demanding that vaccination rates improve to 90% by 2020, and various Medical Societies are recommending mandatory vaccination programs (i.e., requirement for employment).
- To improve influenza vaccination coverage of HCP in Louisiana hospitals we must first understand what is being done, what is effective and what is ineffective.

Objectives

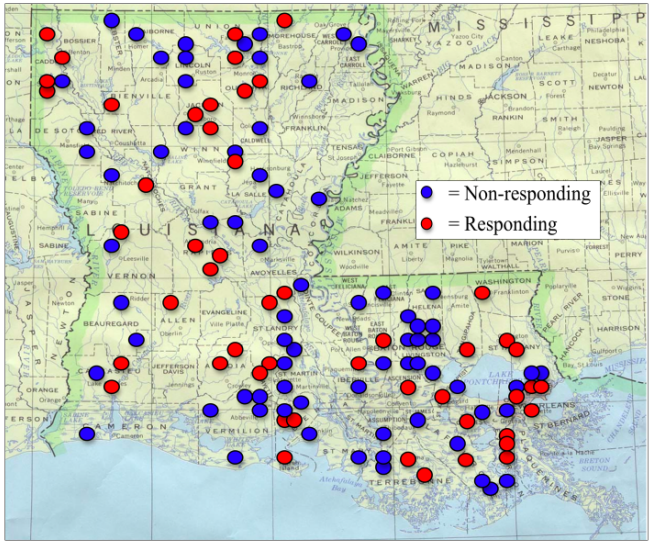
1. To determine influenza vaccination requirements and policies among hospitals in Louisiana, including the prevalence of mandatory requirements and consequences for declination
2. To correlate specific requirements with vaccination rates achieved, and to identify interventions that may increase vaccination rates

Methods

- A survey was sent to all 256 hospitals in Louisiana (under 193 organizations) identified in the Directory of the Louisiana Hospital Association.
- The survey contained questions on type of hospital, patient population served, components of the vaccination program and their estimated vaccination rate.
- Data was inputted into an Excel sheet and analyzed for components that influenced vaccination rates.
- Univariant analysis of categorical data compared the median vaccination rate between hospitals with or without a specific variable using the non-parametric Mann-Whitney test.
- The effect of continuous variables on the vaccination rate was analyzed with regression analysis using the non-parametric Spearman r.
- A p Value of <0.05 was considered significant.

Results: Hospitals Responding

- In the first 4 weeks, 49 (25%) of the 193 administrations responded with a statewide distribution (Figure 1).



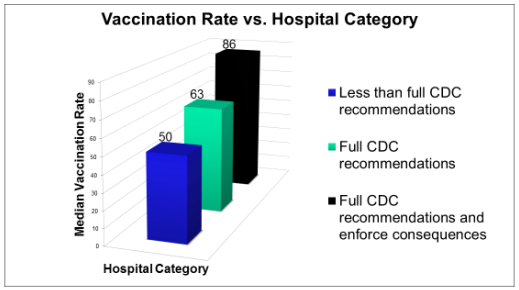
Results: Main Responses

- Most hospitals were private for profit (51%), private non-profit (35%), and public (14%); 22% were teaching and 51% were accredited by The Joint Commission.
- The median number of beds was 60 with a range of 10 – 800.
- All hospitals had a flu vaccination program; 33% had voluntary vaccination and 67% required a formal declination. No hospital demanded vaccination as a requirement of employment.
- All hospitals offered free vaccines; 27% met all CDC recommended activities for vaccination but 73% did not meet all CDC recommendations.
- 24% of hospitals enforced consequences to HCP declining vaccination while 76% had no consequences; the most common consequence was a requirement to wear a mask on patient contact.
- The median vaccination rate reported by the responding hospitals was 61%, with a range from 12 - 98%.

Results: Correlates of Vaccination

Factors Positively Associated with Vaccination Rates

Survey Questions		No. Responses	%	Not Present	Present	Ratio	p Value
				Median (25%, 75%)			
Hospital Type							
	Private	18	37	55 (45, 72)	73 (58, 84)	1.33	0.02
	Acute Care	28	58	50 (45, 72)	70 (57, 81)	1.40	0.02
High-Risk Patient Type							
	Children	29	59	50 (45, 71)	70 (56, 85)	1.40	0.02
	Pregnant Women	23	47	51 (45, 71)	72 (60, 85)	1.41	0.004
	Intensive Care	26	53	50 (42, 70)	71 (57, 85)	1.42	0.006
Number of Beds							
	0 - 99	26	53		50 (45, 71)	0.694	0.0006
	100 - 299	12	24		70 (56, 80)	1.186	
	≥ 300	8	16		85 (61, 92)	1.466	
Vaccination Program							
	Voluntary	16	33	71 (52, 85)	52 (40, 57)	0.73	0.001
	Declination Required	33	67	52 (40, 57)	71 (52, 85)	1.37	0.001
Vaccine Administration							
	Common areas	31	63	48 (37, 52)	70 (59, 83)	1.46	0.001
	Nights/Weekends	38	78	50 (35, 60)	70 (53, 84)	1.40	0.006
Program Promotions							
	Fliers	37	76	43 (33, 56)	69 (55, 80)	1.60	0.005
	Email	34	69	50 (45, 71)	66 (54, 84)	1.32	0.05
Consequences upon Declination							
	None	37	76	86 (82, 93)	55 (45, 70)	0.64	0.0001
	Some consequence	12	24	55 (45, 70)	86 (82, 93)	1.56	0.0001
	Wear mask	10	20	56 (46, 70)	89 (85, 94)	1.59	0.0001



Conclusions

- Preliminary results demonstrate large variability among influenza vaccination programs in Louisiana hospitals. No hospital required vaccination as a condition of employment.
- Hospitals that impose consequences for vaccine declination have a higher vaccination rate than hospitals without consequences.
- Our findings suggest that to reach the goal of 90% vaccination rate by 2020, programs with consequences for declination (e.g. wearing a mask) must be enforced.
- These findings have important public health implications.

Examining Louisiana Mammography Facilities for Medicaid Coverage Gaps

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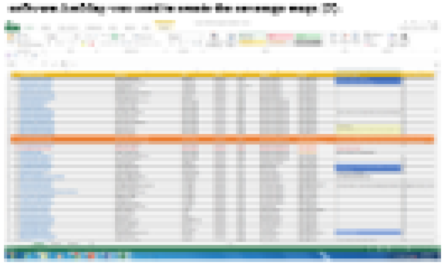
Xavier University of Louisiana[1], Louisiana State University School of Public Health, Louisiana Breast and Cervical Health Program[2]

LSU Health New Orleans School of Medicine

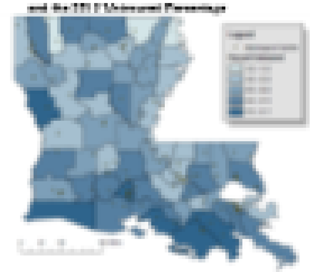
Entergy

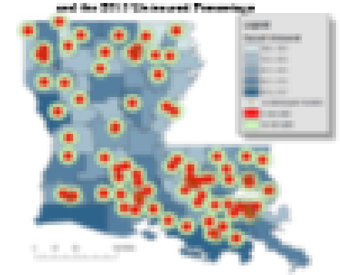
Introduction

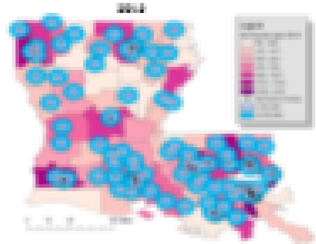
Methods



Results







Conclusions

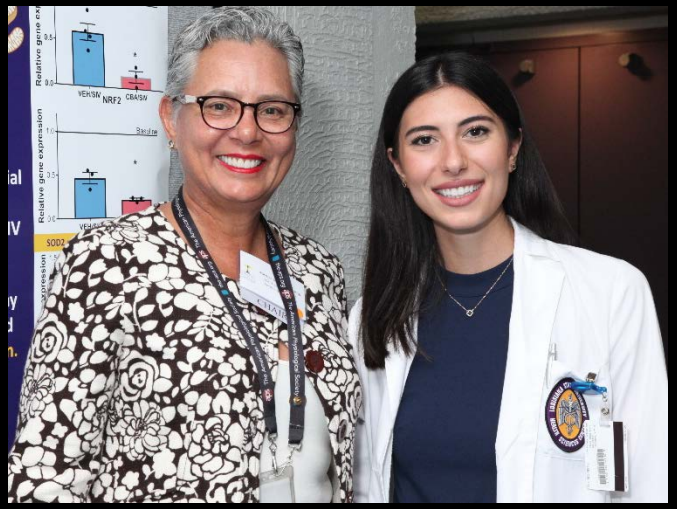
Next Steps

References

This research was supported by the Entergy Workforce Training Grant

Important Deadlines #4, #5,and #6:

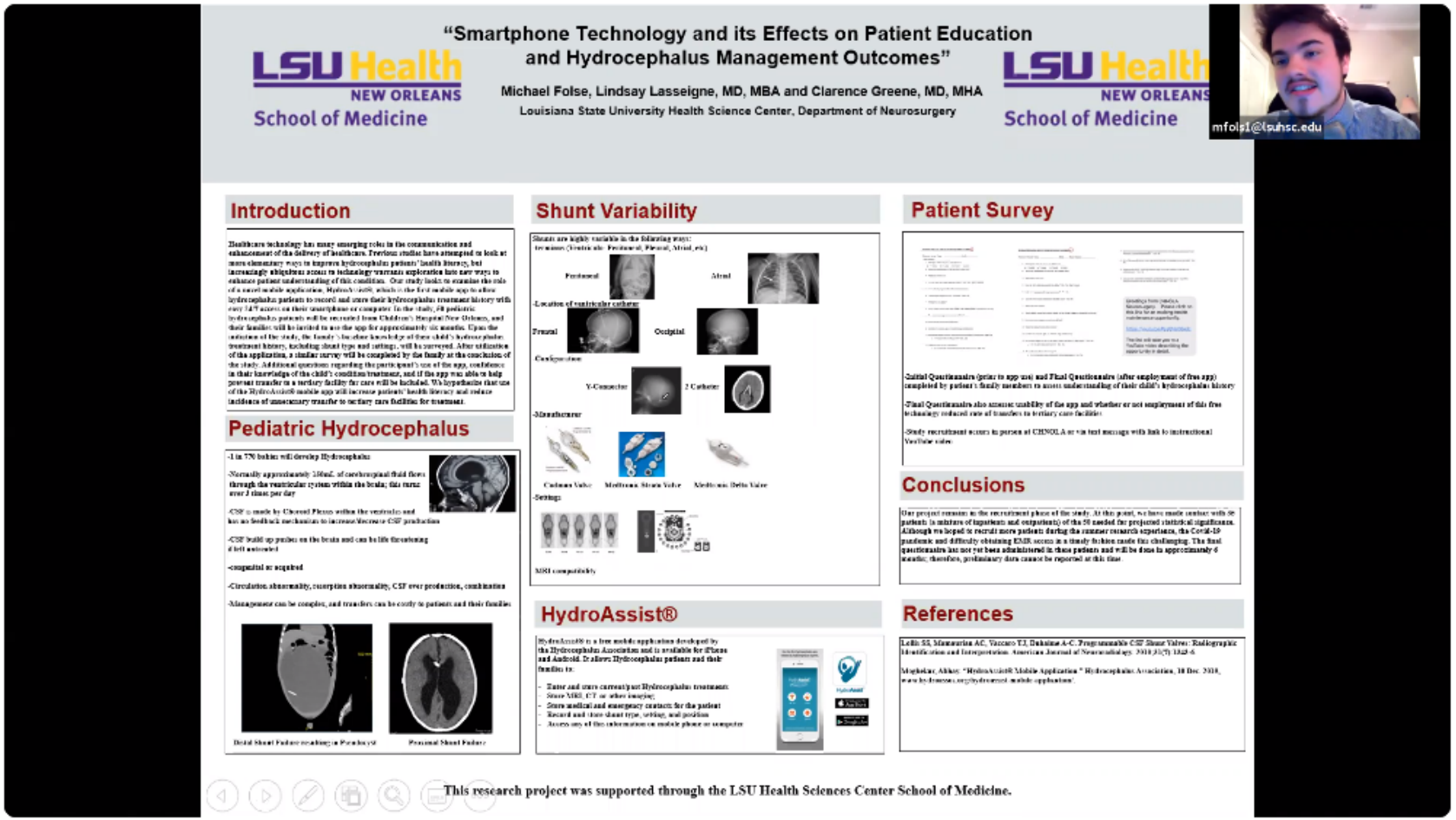
- Record your presentation using Zoom.
- Explain the components of your poster as a 3-5 minute PowerPoint presentation while sharing the screen.
- Send the link of the Zoom recording by Friday, October 16th.
- Also send us your photo by Friday, October 16th.
- We will populate the website with the abstracts, posters, recordings, and your photo
- The judges will receive the abstract book and your pre-recorded presentation on October 26th, and you will have to answer questions using Zoom.
- Check your email and anticipate about three judges to email you to arrange a Zoom meeting on October 26th to October 29th. You will have to answer questions regarding your poster.



- Practice with your mentor and lab mates.
- After you create your final PowerPoint presentation, record yourself presenting it using Zoom.
- When recording, do not save in the computer hard drive; it's hard to retrieve. Save in the iCloud.

Example:

zoom mfols1@lsuhsc.edu's Zoom Meeting - Shared screen with speaker view



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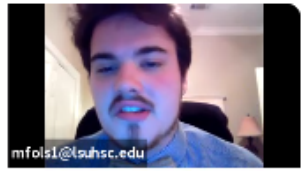
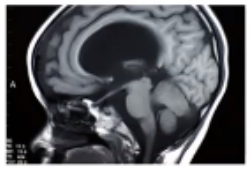
mfols1@lsuhsc.edu's Zoom Meeting - Shared screen with speaker view



Pediatric Hydrocephalus



-1 in 770 babies will develop Hydrocephalus

-Normally approximately 150mL of cerebrospinal fluid flows through the ventricular system within the brain; this turns over 3 times per day

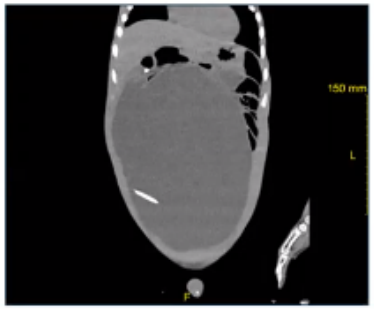
-CSF is made by Choroid Plexus within the ventricles and has no feedback mechanism to increase/decrease CSF production

-CSF build up pushes on the brain and can be life threatening if left untreated

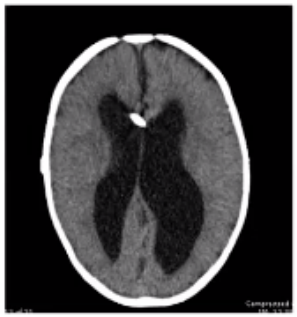
-congenital or acquired

-Circulation abnormality, resorption abnormality, CSF over production, combination

-Management can be complex, and transfers can be costly to patients and their families

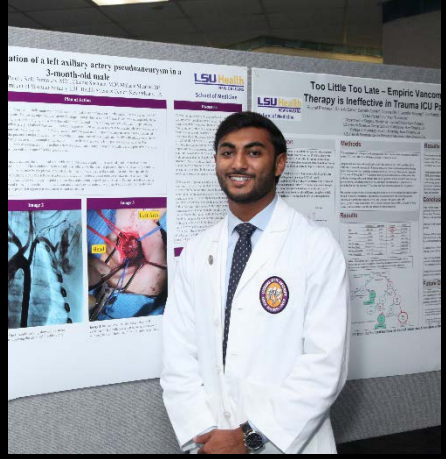


Distal Shunt Failure resulting in Pseudocyst



Proximal Shunt Failure

Next: Practice your presentation

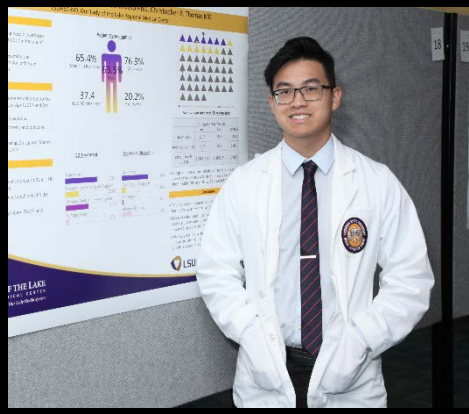


- Practice with your mentor and your lab members!
- Anticipate questions and look up the answers ahead of time
- Practice, practice, practice so you sound polished.
- Practice in front of your friends or in front of a mirror.

What happens at a virtual poster session?

- For your Zoom recording which will be on the website, please dress appropriately (business attire or lab coat) during the presentation
- The abstracts and posters will be available to the public.
- The abstracts and recorded posters will be sent to the judges October 26th

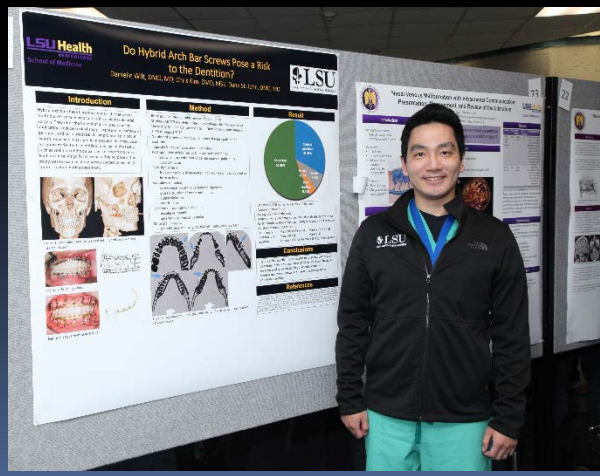
What happens during the judging at a virtual poster session?



- The posters will be judged on the actual poster display and your presentation (enthusiasm, understanding of the topic, etc.)
- Sometimes people ask specific questions, or sometimes they ask "tell me about your project" so prepare a short recap of your project
- Think of possible questions you may be asked. If you do not know an answer, it is OK to say "I don't know"

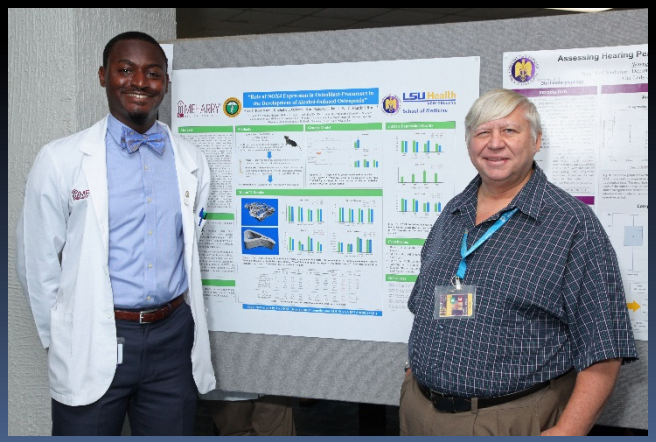
How to deal with nervousness

- Slow down. Take a few seconds to think about a question that is being asked before you answer it.
- Bring notes. if you are afraid that you will forget a point, write it down on a piece of paper and bring it with you.
- However, you don't want to have a verbatim copy of your talk; instead write down key phrases that you want to remember to say.



How to deal with nervousness

- Be prepared to answer questions. You don't have to know the answer to every question, however you should be prepared to answer questions about your work. Before the poster session, think about what questions you are likely to get, and how you would answer them



Zoom Presentation example:

Molecular Cytogenetic Characterization of RH4 and RH30 Alveolar Rhabdomyosarcoma (ARMS) Cell Lines

LSU Health NEW ORLEANS
School of Medicine
Department of Genetics

Jorge Peñas[1], Katrina Gleditsch[1,2], Danielle Mercer[1], Ayesha Umrigar[1], Yuwen Li[3], Tian-Jian Chen[3], Andrew Hollenbach[1], Fern Tsien[1]

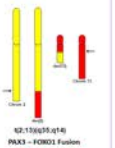
[1] Louisiana State University Health Sciences Center, Department of Genetics, [2] Children's Hospital of New Orleans, [3]Tulane School of Medicine Hayward Genetics Center

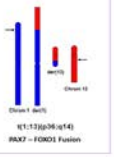
Tulane University SCHOOL OF MEDICINE

Background

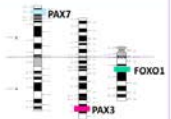
Rhabdomyosarcoma is the most common soft tissue sarcoma in children. More than half of childhood rhabdomyosarcomas are diagnosed in those under the age of 10. The two most common subtypes of rhabdomyosarcoma are alveolar (ARMS) and embryonal (ERMS).

- ERMS makes up 60% of rhabdomyosarcoma cases
- ARMS makes up 30% of cases and typically has a more aggressive clinical course and portends a poorer clinical prognosis.
- Details on ARMS:
  - Histologically distinct
  - Genetically characterized by balanced translocations at t(2;13) or t(1;13).
  - Translocations lead to the production of fusion proteins PAX3-FOXO1 t(2;13) and PAX7-FOXO1 t(1;13)
  - PAX3-FOXO1 variant of ARMS is known to be more aggressive.
  - Current diagnostic break apart probe cannot distinguish between translocations





Our laboratory, in collaboration with Cytocell Ltd., designed fluorescence *in situ* hybridization (FISH) probes specific for the PAX3-FOXO1 and PAX7-FOXO1 translocations, which allows for a more accurate diagnosis by differentiating between t(1;13) and t(2;13).



Two of the most widely used commercially available cell lines used in ARMS research are RH4 and RH30. These cell lines were derived from pediatric patients clinically diagnosed with ARMS. Previous studies have cytogenetically characterized the RH30 cell line to gain a better understanding of phenotypic clinical correlations due to these translocations; however, to date the RH4 cell line has not been fully cytogenetically characterized.

Table 1: Characteristics of RH4 and RH30 cell lines

	RH4	RH30
Age	7 Years Old	16 Years Old
Sex	Female	Male
Origin	Lung metastasis	Bone Marrow
Gene Fusion	t(2;13)	t(2;13)

The specific aims of this project are to 1) cytogenetically characterize the RH4 and RH30 cell lines since these cell lines are used in clinical research where cytogenetic variants can affect clinical outcomes and to 2) validate our newly designed ARMS probes.

These experiments are also being preformed on patient derived ARMS tumor samples thus further validating the newly designed probes to determine their clinical diagnostic utility. **(See Gleditsch, *et al.* poster #2818)**

Methods

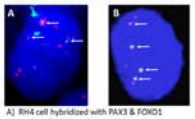
Table 2: Description and brief protocol of methods used to validate the newly designed probes

Methods	
aCGH+SNP (array Comparative Genomic Hybridization + Single Nucleotide Polymorphism)	Array CGH was preformed on SurePrint G3 Human CGH+SNP 4x180k array slides, scanned by Agilent DNA Microarray Scanner with SureScan High-Resolution, and analyzed by Agilent FeatureExtraction and Cytogenomics.
G-Banding (Giemsa Banding)	Chromosomes were harvested from RH30, RH4 and normal cells using colcemid, 0.075 M KCl , and Carnoy's fixative (3:1 Methanol: Glacial Acetic Acid). Slides were G-banded using trypsin and Giemsa stain. Slides were analyzed at 100X using a bright field microscope.
SKY (Spectral Karyotyping)	Chromosomes were prepared as above. SKY was performed using 24 chromosome-specific probes (Molecular Cytogenetics Core Facility, MD Anderson Cancer Center) and analyzed at 100X with a fluorescent microscope.
FISH (Fluorescence *in situ* Hybridization)	Chromosomes were prepared as above and hybridized with newly designed probes specific for PAX3, FOXO1, and PAX7. These probes were labeled with Spectrum Orange, FITC, & Spectrum Aqua, respectively, followed with a DAPI counterstain, and analyzed at 100X using a fluorescent microscope.

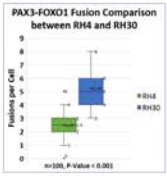
Results

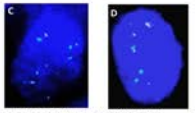
FISH

Figure 1: ARMS probes used on RH4 and RH30 cell lines.

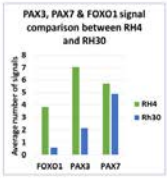


A) RH4 cell hybridized with PAX3 & FOXO1 probes. Fusions pointed out by arrow. (3 Shown)
B) RH30 cell hybridized with PAX3 & FOXO1 probes. Fusions pointed out by arrow. (4 Shown)



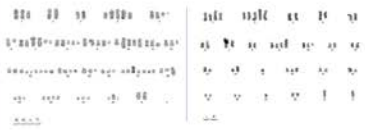


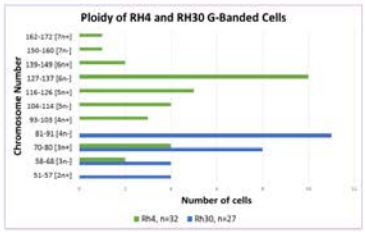
C) RH4 cell hybridized with PAX7 & FOXO1 probes. Images demonstrate amplification of PAX7 and FOXO1 in both cell lines.
D) RH30 PAX7 & FOXO1 probes. Images demonstrate amplification of PAX7 and FOXO1 in both cell lines.



Karyotype G-banding

Figure 2: Karyotype (Left: RH4, Right: RH30)





SKY (RH4)

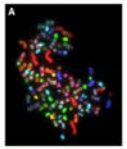
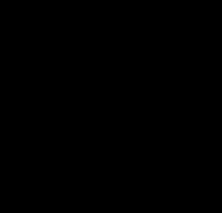


Figure 3: Spectral karyotyping of one representative cell from RH4 cell line. **A)** Metaphase spread **B)** Organized karyotype demonstrates aneuploidy and multiple chromosomal rearrangements. Up to 5 rearrangements are shown in a single chromosome. Each chromosome is displayed in triplicate: G-Banded, fluorescent, and a computer assigned color, respectively.

aCGH

Figure 4: arrayCGH and expanded regions of chromosome 17.



ArrayCGH of **A)** RH4 and **B)**RH30 cells. Expanded regions of Chromosome 17. Purple box highlights 17p13.1 (TP53) in **C)**RH4 and **D)**RH30

Table 2: Copy number variations (CNV) between RH4 and RH30 cell lines determined by array Comparative Genomic Hybridization (arrayCGH).

Gene	Location	Function	RH4	RH 30
NRAS	1p13.2	Provides instructions for making a protein that is involved primarily in regulating cell division.	+	O
MYCN	2p24.1	Proto-oncogene member of the MYC family of transcription factors.	O	+
MET	7q31.2	Mesenchymal Epithelial Transition MET is a prototypical receptor tyrosine kinase.	O	+
CDK4	12q13.3	Involved in cell cycle progression.	O	+
TP53	17p13.1	Codes for a protein that regulates the cell cycle and functions as a tumor suppressor.	-	-
MDM2	12q14.3	Encodes a nuclear-localized E3 ubiquitin ligase. Target tumor suppressor proteins, such as p53.	+	O
PAX3	2q35	These genes play critical roles during fetal development. Necessary for myogenesis.	+	O
PAX7	1p36.13	Transcription factor playing a role in myogenesis through regulation of muscle precursor cells proliferation.	+	
FOXO1	13q14.1	Forkhead family of transcription factors. Play a role in myogenic growth and differentiation.	O	+

Discussion

This study provides the cytogenetic and molecular genetic characterization of commonly used ARMS cell lines, RH4 and RH30. The cell lines were analyzed using G banding, fluorescence *in situ* hybridization (FISH), array comparative genomic hybridization (aCGH) and spectral karyotyping (SKY).

FISH results using our newly developed probes specific for PAX3, PAX7, and FOXO1 showed that RH30 had more PAX3-FOXO1 fusions when compared to RH4. However, RH4 had a significant amplification of PAX3, PAX7, and FOXO1.

Consistent with our FISH results, G-Banding demonstrated a significant difference in ploidy between RH4 and RH30. Aneuploidy for RH4 ranged from 59-163 chromosomes, whereas RH30 had 51-90 chromosomes.

Initial SKY analysis of RH4 shows large scale and highly complex chromosomal rearrangements and aneuploidy throughout the genome.

ArrayCGH showed similarities and differences in chromosomal amplifications and deletions between the two cell lines. Genes within similar regions were selected from previously published reports with known association to rhabdomyosarcoma. The amplification of PAX3 in RH4 seen in our aCGH is consistent with our FISH results, which serves as an internal positive control.

While both cell lines originate from patients diagnosed with ARMS, they exhibit significant cytogenetic variations, highlighting the necessity of genetic characterization to direct potential targeted treatment options.

Our future aims for this study is to further characterize the genetic variation among ARMS cell lines and patient tumors with bioinformatics and pathways analysis software. Furthermore, RNA sequencing and epigenetic analysis will be preformed on ARMS tumor samples.

Currently the second arm of our study is looking at the heterogeneity of 2;13 translocations in ARMS patients at hospitals in New Orleans further validating our newly designed FISH probes. **(See Gleditsch, *et al.* poster #2818)**

Molecular Cytogenetic Characterization of RH4 and RH30 Alveolar Rhabdomyosarcoma (ARMS) Cell Lines

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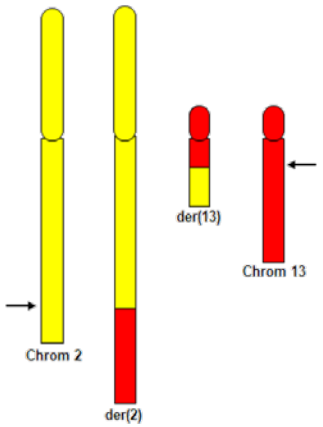
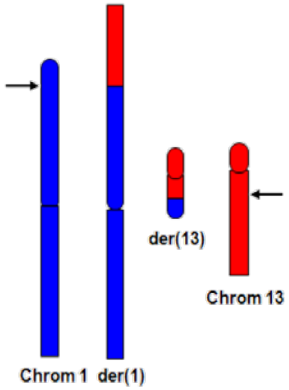


t(2;13)(q35;q14)

PAX3 – FOXO1 Fusion

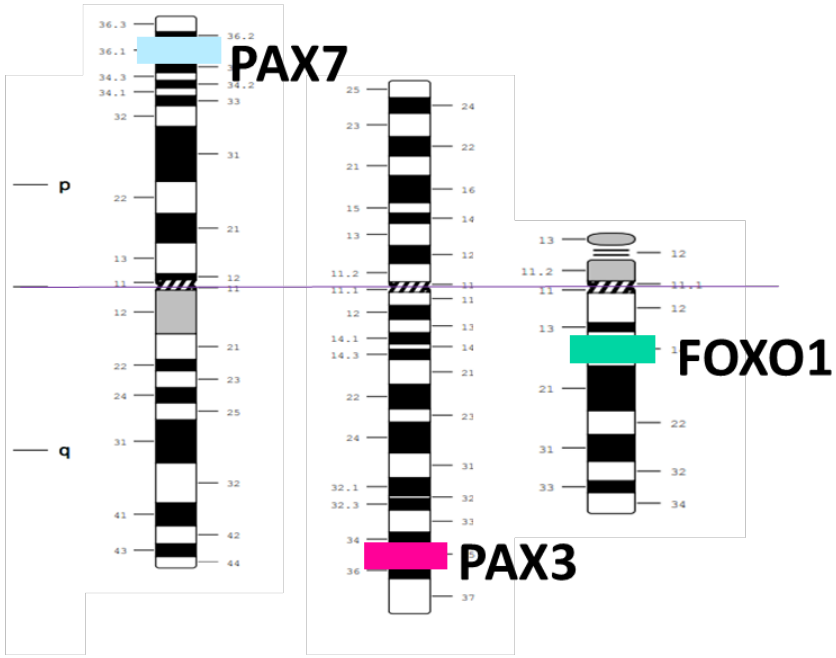
More Aggressive,
Worse Prognosis



t(1;13)(p36;q14)

PAX7 – FOXO1 Fusion

Our laboratory, in collaboration with Cytocell Ltd., designed fluorescence *in situ* hybridization (FISH) probes specific for the PAX3-FOXO1 and PAX7-FOXO1 translocations, which allows for a more accurate diagnosis by differentiating between t(1;13) and t(2;13).



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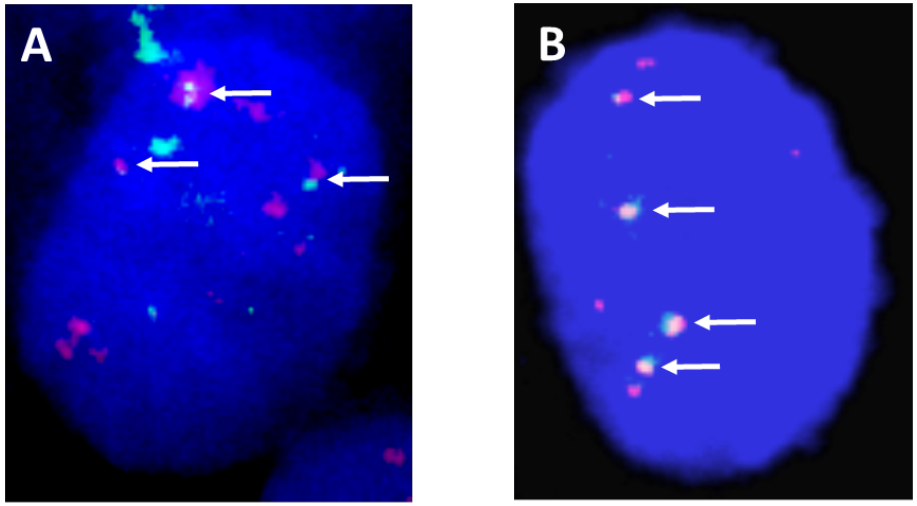
Results

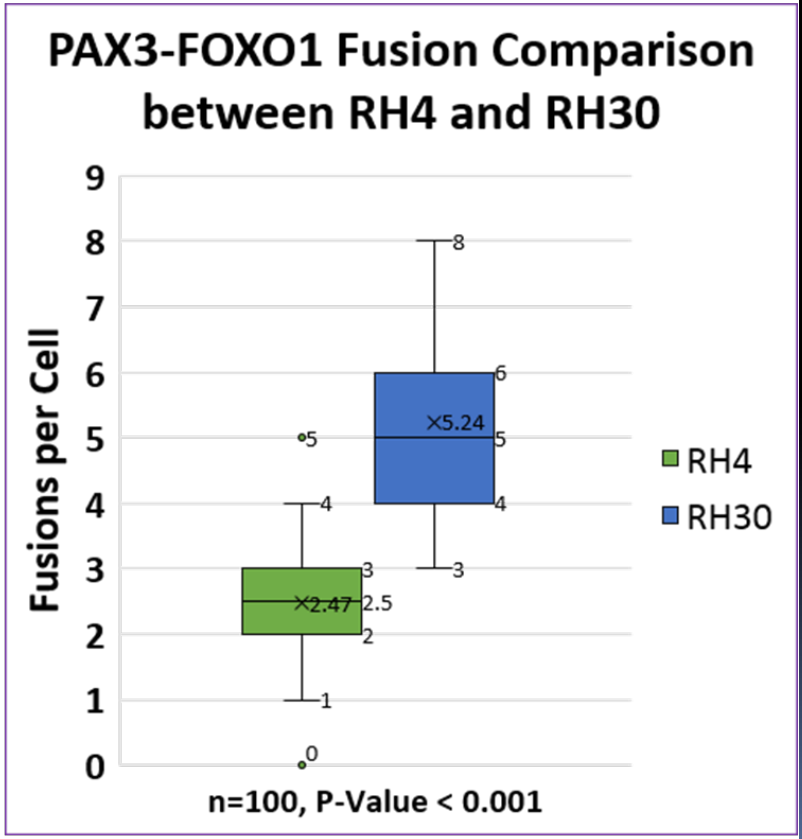
FISH

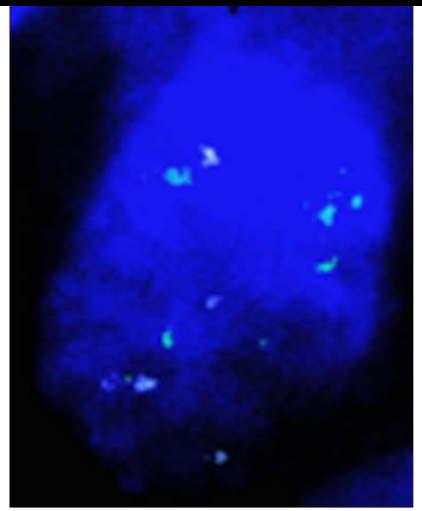
Figure 1: ARMS probes used on RH4 and RH30 cell lines.

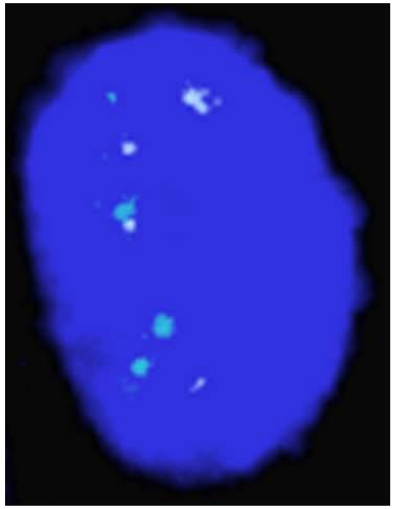


A) RH4 cell hybridized with PAX3 & FOXO1 probes. Fusions pointed out by arrow. (3 Shown)

B) RH30 cell hybridized with PAX3 & FOXO1 probes. Fusions pointed out by arrow. (4 Shown)

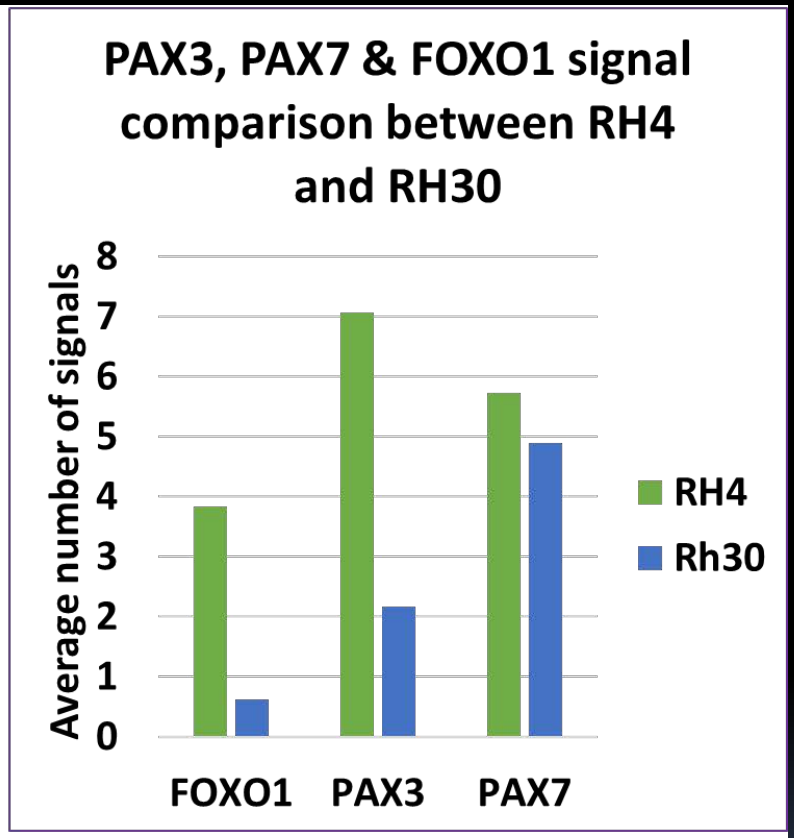






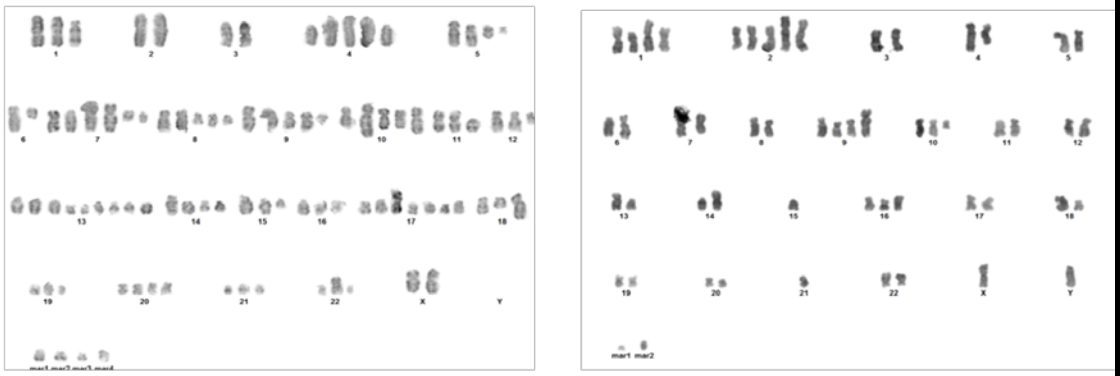
C) RH4 cell hybridized with PAX7 & FOXO1 probes. Images demonstrate amplification of PAX7 and FOXO1 in both cell lines.

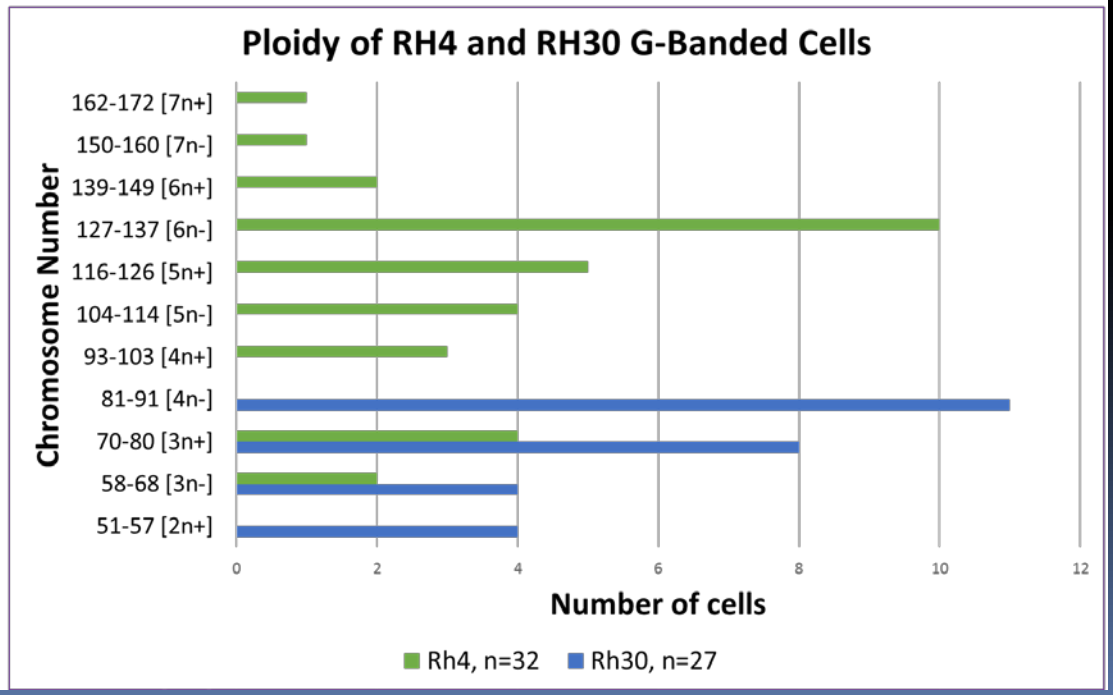
D) RH30 PAX7 & FOXO1 probes. Images demonstrate amplification of PAX7 and FOXO1 in both cell lines.



Karyotype G-banding

Figure 2: Karyotype (Left: RH4, Right: RH30)





SKY (RH4)

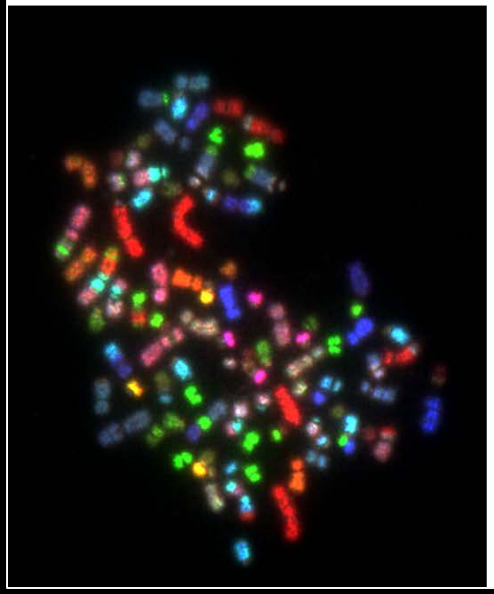
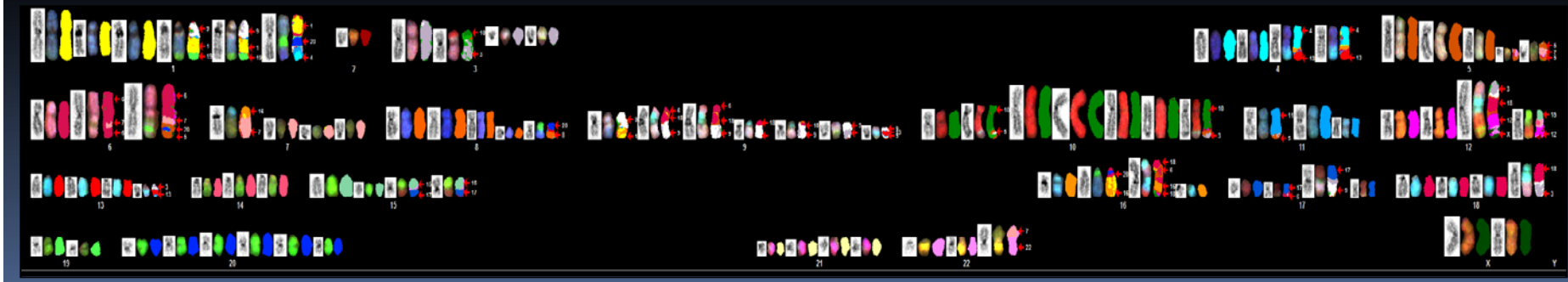
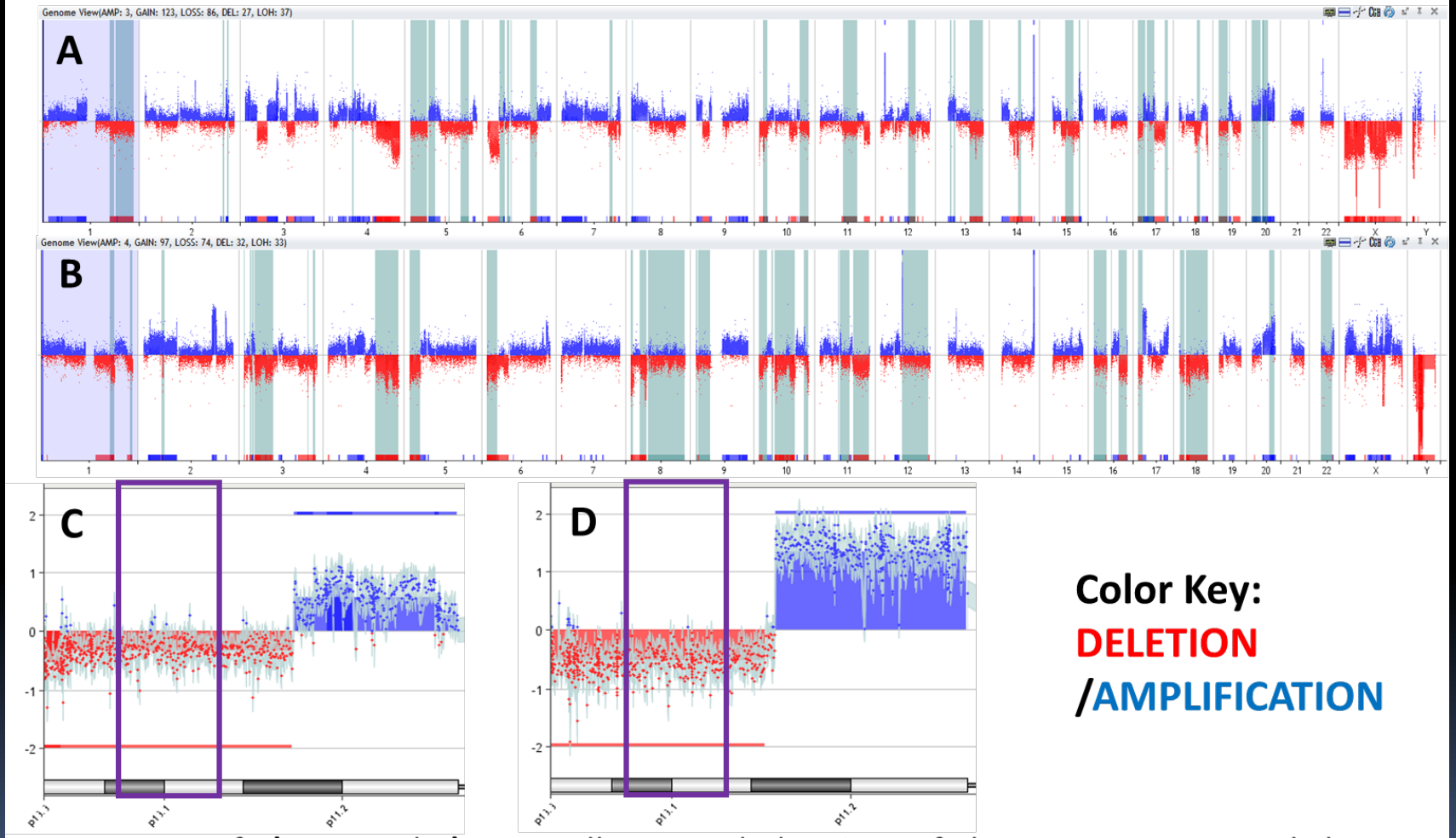


Figure 3: Spectral karyotyping of one representative cell from RH4 cell line. **A)** Metaphase spread **B)** Organized karyotype demonstrates aneuploidy and multiple chromosomal rearrangements. Up to 5 rearrangements are shown in a single chromosome. Each chromosome is displayed in triplicate: G-Banded, fluorescent, and a computer assigned color, respectively.



aCGH

Figure 4: arrayCGH and expanded regions of chromosome 17.



ArrayCGH of **A)** RH4 and **B)**RH30 cells. Expanded regions of Chromosome 17. Purple box highlights 17p13.1 (TP53) in **C)**RH4 and **D)**RH30

Table 2: Copy number variations (CNV) between RH4 and RH30 cell lines determined by array Comparative Genomic Hybridization (arrayCGH).

Gene	Location	Function	RH4	RH 30
NRAS	1p13.2	Provides instructions for making a protein that is involved primarily in regulating cell division.	+	0
MYCN	2p24.1	Proto-oncogene member of the MYC family of transcription factors.	0	+
MET	7q31.2	Mesenchymal Epithelial Transition MET is a prototypical receptor tyrosine kinase.	0	+
CDK4	12q13.3	Involved in cell cycle progression.	0	+
TP53	17p13.1	Codes for a protein that regulates the cell cycle and functions as a tumor suppressor.	-	-
MDM2	12q14.3	Encodes a nuclear-localized E3 ubiquitin ligase. Target tumor suppressor proteins, such as p53.	+	0
PAX3	2q35	These genes play critical roles during fetal development. Necessary for myogenesis.	+	0
PAX7	1p36.13	Transcription factor playing a role in myogenesis through regulation of muscle precursor cells proliferation.	+	-
FOXO1	13q14.1	Forkhead family of transcription factors. Play a role in myogenic growth and differentiation.	0	+

Discussion

This study provides the cytogenetic and molecular genetic characterization of commonly used ARMS cell lines, RH4 and RH30. The cell lines were analyzed using G banding, fluorescence *in situ* hybridization (FISH), array comparative genomic hybridization (aCGH) and spectral karyotyping (SKY).

FISH results using our newly developed probes specific for PAX3, PAX7, and FOXO1 showed that RH30 had more PAX3-FOXO1 fusions when compared to RH4. However, RH4 had a significant amplification of PAX3, PAX7, and FOXO1.

Consistent with our FISH results, G-Banding demonstrated a significant difference in ploidy between RH4 and RH30. Aneuploidy for RH4 ranged from 59-163 chromosomes, whereas RH30 had 51-90 chromosomes.

Initial SKY analysis of RH4 shows large scale and highly complex chromosomal rearrangements and aneuploidy throughout the genome.

ArrayCGH showed similarities and differences in chromosomal amplifications and deletions between the two cell lines. Genes within similar regions were selected from previously published reports with known association to rhabdomyosarcoma. The amplification of PAX3 in RH4 seen in our aCGH is consistent with our FISH results, which serves as an internal positive control.

While both cell lines originate from patients diagnosed with ARMS, they exhibit significant cytogenetic variations, highlighting the necessity of genetic characterization to direct potential targeted treatment options.

Our future aims for this study is to further characterize the genetic variation among ARMS cell lines and patient tumors with bioinformatics and pathways analysis software. Furthermore, RNA sequencing and epigenetic analysis will be preformed on ARMS tumor samples.

Currently the second arm of our study is looking at the heterogeneity of 2;13 translocations in ARMS patients at hospitals in New Orleans further validating our newly designed FISH probes. (**See Gleditsch, *et al.* poster #2818**)

Refer to this website as an example:

- https://www.medschool.lsuhsc.edu/genetics/2020_summer_internship_poster_session.aspx

Questions?
Remember:

1. Let us know you are participating: September 30th
2. Abstracts due: October 5th
3. Posters due: October 9th
4. Recording due: October 16th
5. Your photo due: October 16th
6. Zoom appointments with three judges:

   October 26, 27, 28, or 29th (date and time: judges will arrange with students)

5. October 30th: We will announce the winners (L1/L2 category and L3/L4 category) , who will receive travel awards to present their research at national conferences.