HOW TO PRESENT AT A SCIENTIFIC MEETING

Dr. Fern Tsien
Department of Genetics
LSUHSC

Important Deadline #1:

- Abstracts are due on or before Friday, July 18th by 5:00!!!
- Medical student abstracts are due on August 1st.
- Follow the guidelines sent to you.
- DO NOT change the font size or style.
- We will use the abstract you send us to generate the Abstract Book to give out during the poster session and to the judges ahead of time.

What is an Abstract?

- An abstract is a one-page summary of your project.
- List your name, mentor's name, etc. as described in the template.
- Affiliations: department and school
- Use only the template we provide.
- 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, please be sure to save the file with your last name listed first. For example: ShieldsHeatherAbstract.docx
- Send it to: Wanda Joseph wjose3@lsuhsc.edu

AND Brianne Jones bjon13@lsuhsc.edu

Your Name (first, middle initial, last)
Classification (High School, Undergraduate, Medical)
Name of School, City, State

Mentor's Name:
Mentor's Affiliation (LSUHSC, Tulane SOM, Xavier, Children's Hospital, etc.)

"Title of Project"

Abstract (summary of project, not to exceed one page)

Body of Abstract: Left Justified, 11 pt Arial font.

Charity F. Sylvester
Undergraduate
Xavier University, New Orleans, LA

Mentor: Imran Mungrue, Ph.D.
Louisiana State University Health Sciences Center, Department of Pharmacology and Experimental Therapeutics

"Assessing SNPs in the ABCC6 Transporter in an Acadian Family Predisposed to Cardiovascular Disease"

Cardiovascular disease (CVD) encompasses pathologies of the cardiovascular system, which includes diseases of the heart and arteries. Many factors, including genetics, behavior, ethnicity and environment are known to contribute to the disease progression. However, only about 10% of the genetic causes of CVD have been defined. Pseudoxanthoma elasticum (PXE) is a genetic disorder that causes calcified skin lesions known as pseudoxanthomas, retinal deterioration, or expedited arteriosclerosis. PXE has been linked to a mutation in ABCC6, which has also been associated with an increase in coronary artery disease. We found an Acadian family predisposed to premature cardiovascular disease, with eight family members experiencing myocardial infarction prior to age 43. A genetic cause for this familial aggregation is not yet known. We hypothesized that a SNP in the gene ABCC6 (ATP Binding Cassette Subfamily C Member 6) could contribute. The function of ABCC6 is currently unknown.

In our study, we sought to determine whether members of the family possessed a SNP, (rs726537060), which results in a nonsense mutation in ABCC6 in which arginine is substituted for a termination amino acid at codon 1141. The alleles associated with this SNP are cytosine (C) and thymine (T). Cytosine is the major allele and thymine is the pathogenic, minor allele. Thymine has a 3% minor allele frequency. People who are affected by PXE possess a homozygous recessive genotype at the SNP, but studies suggest that a heterozygous genotype can cause symptoms associated with PXE such as premature atherosclerosis.

We found in all the samples studied that they did not posses the pathogenic allele. This means none of the family members studied expressed the pathogenic allele. We conclude that the R1141X SNP in the ABCC6 gene is not a genetic factor causing premature cardiovascular disease in the Acadian family. Further studies will focus on global SNP associations.

[redacted]

[redacted]

Supernumerary marker chromosome (SMC) 17: new case report, delineation of the phenotype, and comparison with other segmental 17p duplications.

Trisomy of the short arm (p arm) of chromosome 17 resulting from a supernumerary marker chromosome (SMC) is very rare producing a variety of phenotypes, with some patients often dying at an early age. We present a 3 week old patient with facial anomalies including cleft palate and cardiac defects. High resolution chromosomes and fluorescence in situ hybridization were done which revealed a trisomy of the 17 p arm and part of q arm (the long arm). We intend to compare this new case with other cases of trisomy 17p and display why our patient is unique.

Important Deadline #2:

- Posters are due Wednesday, July 23rd at 5:00!

If we do not receive a poster by this date, your mentor will be responsible for the printing of the poster!!!

Important deadline #3:

Summer research Internship Poster Day Wednesday, July 30, 2014

- Medical education Building (MEB), 1901 Perdido St, NO, LA 70112

Schedule:

- 12 noon-1:00 pm, First Floor Lobby
  Hang up your poster

- 1-2:30 pm, First Floor Lobby
  Interns and judges only!

- 2:30-3:30, First Floor Lobby
  Open to the public

- 3:30-4:30 First floor Auditorium A
  Awards ceremony, open to the public

Who will be presenting posters?

- All high school and undergraduates in this program are required to present a poster on the 30th.
- Since classes begin early for medical students, they will present their posters during the medical research day in the fall.
- Student presentations will be judged and awards will be given for each category

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!
- Make sure that you understand everything you write on the poster. You should be able to explain your project to the judges.
- In general, try to keep text towards the outside and figures and tables in the center.
- The abstract is not necessary for the poster.

Preparing the posters

- Use the Power Point poster template sent to you by Wanda Joseph or Brianne Jones (not your friends or past interns) with the proper logos.
- These correspond to your mentor's affiliation and the Summer Program 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 depending on the amount of text or figures you have.
- 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 do not know what "DBS" or "FSHD" is.
- Refer to guidelines sent to you.

Once your poster is done:

- Save it as a PPT or PPTX file.
- When you submit your poster, be sure to save the file with your last name listed first. For example: ShieldsHeatherPoster.pptx
- Send it to: Wanda Joseph wjose3@lsuhsc.edu

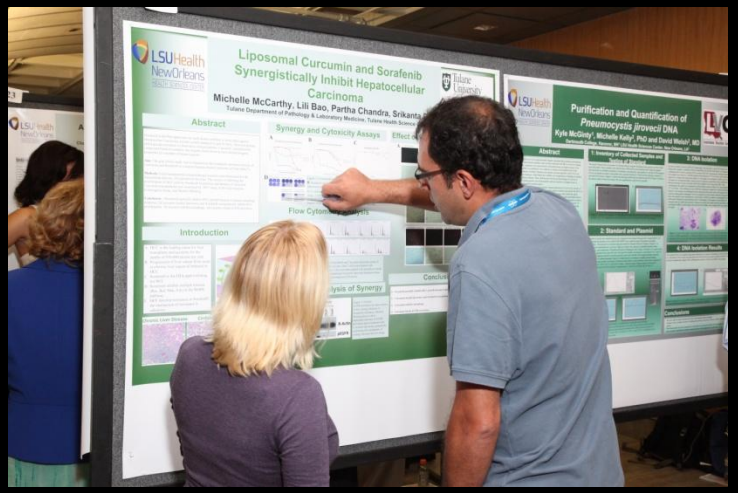
AND Brianne Jones bjon13@lsuhsc.edu

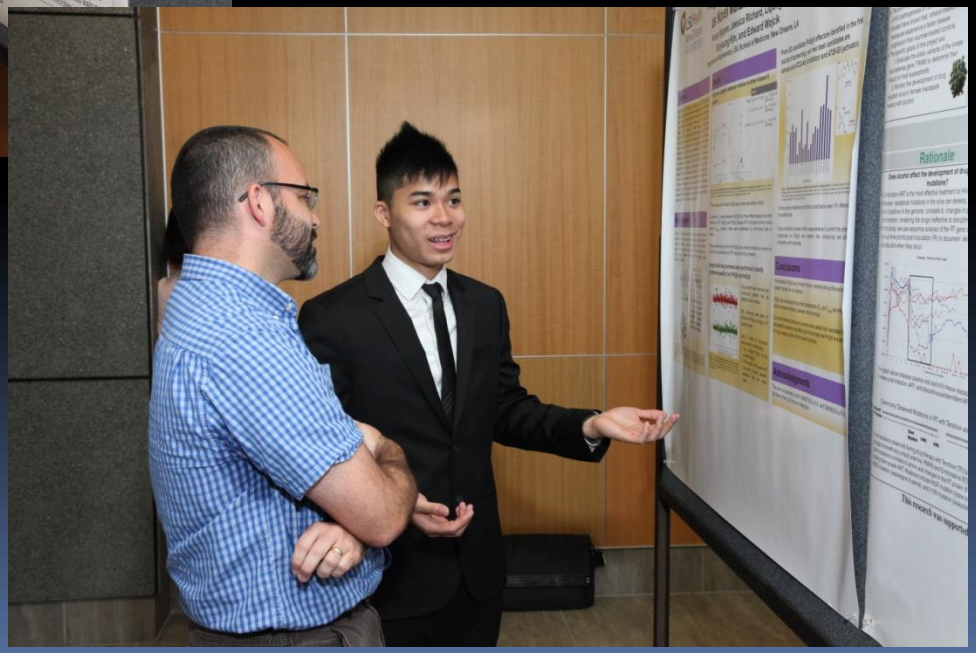
- You will be notified when your poster is ready to pick up from the Genetics office.
- You are responsible for hanging up the poster on July 30th at 12:00.
- Plan to take your poster down at the end of the poster session and give it to your mentor. Let us know in advance if you want an extra one for yourschool.
- Posters are due Wednesday, July 23rd!!!
- If we do not receive a poster by this date, your mentor will be responsible for the printing of the poster.

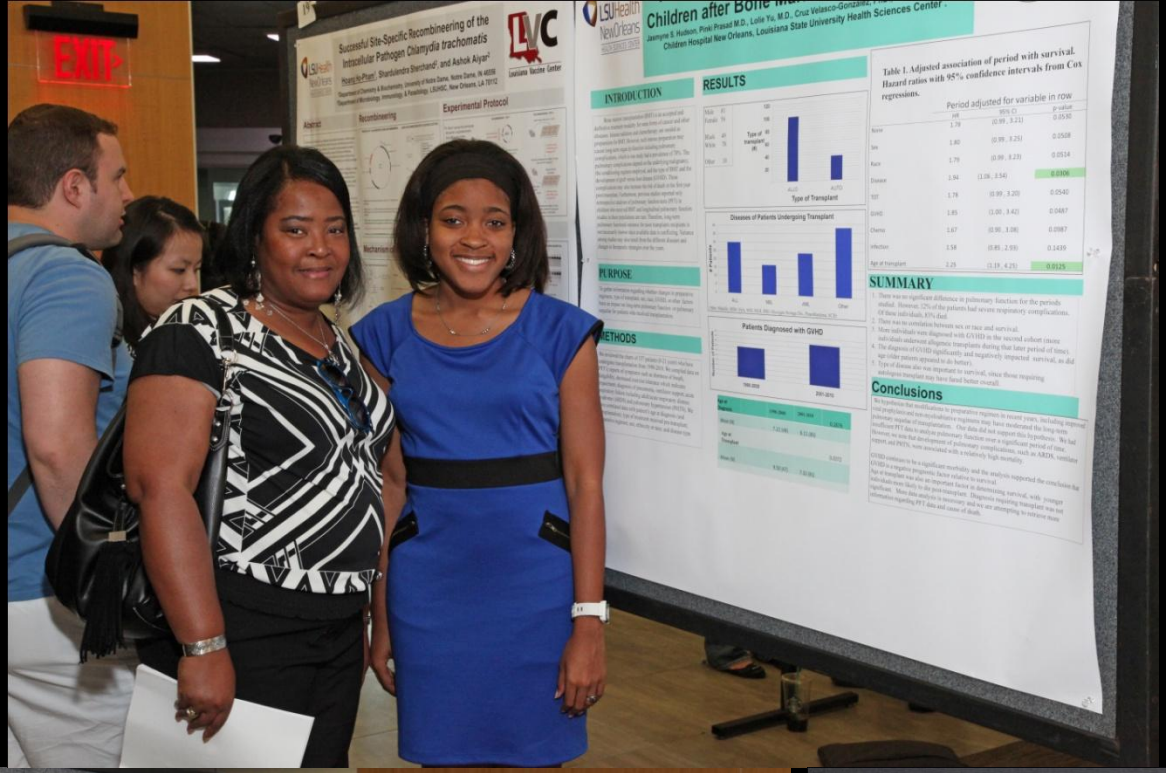
Next: Practice your presentation

- 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 poster session?






EXIT
Successful Site-Specific Recombineering of the Intracellular Pathogen Chlamydia trachomatis
Children after Bone
Children Hospital New Orleans, Louisiana State University Health Sciences Center
INTRODUCTION
RESULTS
PURPOSE
METHODS
SUMMARY
Conclusions


Assessment of Need for Targeted Acute HIV Testing
A New Purpose for an Old Drug: Inhibiting Autophagy with 3-Methyladenine in Ataxia Telangiectasia
Introduction
Cohort Description
Results
Conclusions
Discussion
Acknowledgments

What happens at a poster session?

- Please dress appropriately (business attire).
- The posters will be displayed early so the judges will have a chance to see them ahead of time. Also, they will have a copy of the abstracts.
- At 1:00, stand by your poster. Judges will be passing by asking you questions.
- The posters will be judged on poster display and your presentation (enthusiasm, understanding of the topic, etc)
- Practice ahead of time a short (2-5 minute) description of your poster. Sometimes people ask specific questions, sometimes they ask "tell me about your project"
- DO NOT READ THE POSTER TO THE JUDGES
- Think of possible questions you may be asked. If you do not know an answer, it is OK to say "I don't know"
- At 2:30 the posters will be available to the public. Your family is invited.
- At 3:30 we will move to the 1st floor auditorium and give out awards!

Nervousness: How to fight back

- Practice ahead of time. A well organized, practiced talk will almost always go well.
- If you draw a blank, then looking at your poster will help you get back on track.
- Taking a deep breath will calm you down.
- 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.
- 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
- It is okay to say "I don't know" or "I hadn't thought about that, but one possible approach would be to..."

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

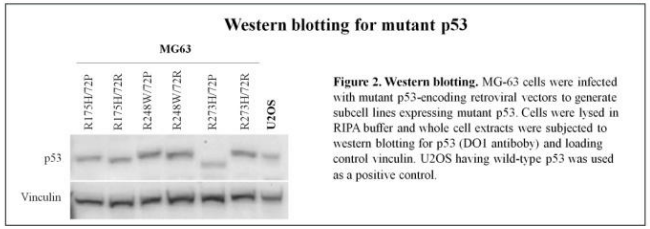


Figure 2. Western blotting. MG-63 cells were infected with mutant p53-encoding retroviral vectors to generate subcell lines expressing mutant p53. Cells were lysed in RIPA buffer and whole cell extracts were subjected to western blotting for p53 (DO1 antiboby) and loading control vinculin. U2OS having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

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.

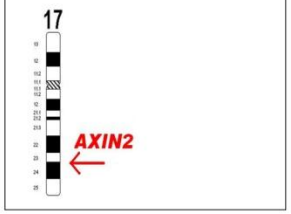
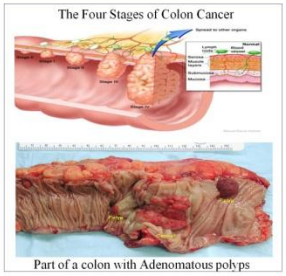


Figure 2



Part of a colon with Adenomatous polyps

Figure 3

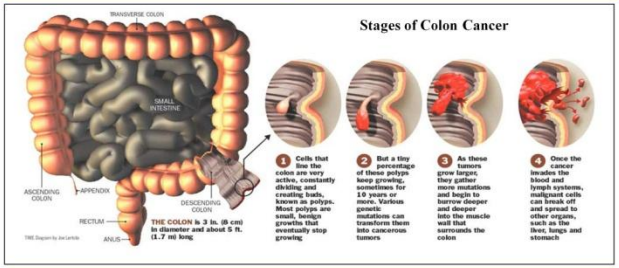
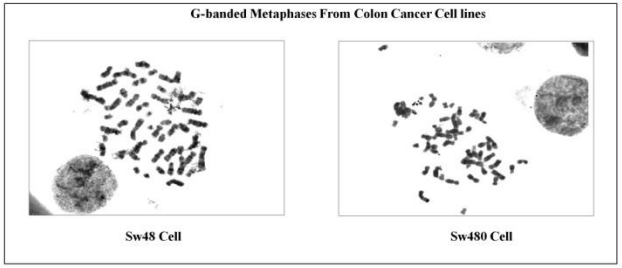


Figure 4



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

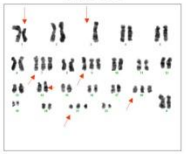
The table to the right shows the frequency of different ploidies in the Sw48 and Sw480 colon cancer cell line.

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%)

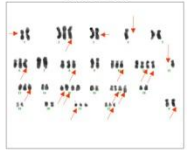
G-Banded Karyotypes Representative of Colon Cancer Cell lines. The Red Arrows indicate abnormalities.

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.

Example of a better poster

LSUHealth NewOrleans HEALTH SCIENCES CENTER

Expression of *Irf-7* in Plasmacytoid Dendritic Cells is Limited Following Neonatal Respiratory Syncytial Virus Infection

Names
Affiliations

Louisiana Vaccine Center

Abstract

Nearly all infants are infected with respiratory syncytial virus (RSV) by two years of age. In infants, RSV is the major cause of bronchiolitis and infants who acquire severe RSV bronchiolitis are at risk of developing asthma. Immune protection is incomplete and reinfection is common throughout life. In otherwise healthy adults, RSV infection usually induces mild upper respiratory tract disease. The mechanisms whereby RSV induces severe disease in infants are largely unknown.

We previously found that neonatal, unlike adult, mice fail to induce appropriate antiviral defenses. In particular, type I interferons are not produced in response to RSV infection. As type I interferons are mainly produced by plasmacytoid dendritic cells (pDCs) via interferon regulatory factor 7 (IRF-7: a transcription factor), we hypothesized that neonatal pDCs in response to RSV infection express less *Irf-7* than adults.

To test this hypothesis, we infected neonatal mice (5d old) and adult mice (7-8wks old) with RSV and purified pDCs from the lung 24h post infection. We then isolated total RNA from the purified pDCs and reverse transcribed the RNA to produce cDNA. Real time PCR was performed with the resulting cDNA to quantify the relative amount of *Irf-7* in neonatal and adult pDCs.

We found that pulmonary pDCs from naïve neonates express seven fold less *Irf-7* than pDCs from adults. When infected with RSV, expression of *Irf-7* in pulmonary pDCs from both neonates and adults increased; however, neonatal pDCs expressed significantly less *Irf-7* than adults. These data indicate that the muted induction of Irf-7 expression in pDCs may play a role in RSV pathogenesis in neonates.

Introduction

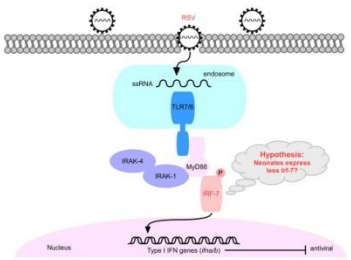


Fig1: RSV induces Type I IFN production in adult pDCs. RSV enters the cells and fuses with endosomal membrane releasing its genomic ssRNA. ssRNA is recognized by host TLR7/8 and induces a cascade of signaling events leading to the phosphorylation of IRF-7. Phosphorylated IRF-7 then translocates to the nucleus and promotes the expression of type I IFNs.

Methods

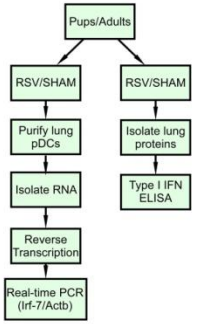


Fig 2: Schematic of the experimental design. Five day old pups or 6-8 wks old adults were infected with RSV or sham infected with media. At one day post infection, total protein was isolated from the lungs of half of the mice. Type I IFNs were measured in the isolated protein using ELISA. The other half of the mice were used for lung pDC purification. RNA were isolated from these purified pDCs and reverse transcribed to cDNA. The resulting cDNA were subjected to real-time PCR to measure the expression of *Irf-7* in pDCs.

Purity of the Isolated pDCs

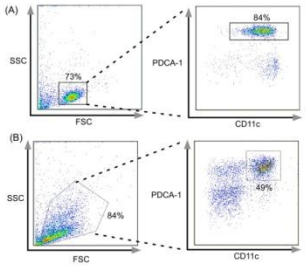


Fig 3: Purity of the isolated pDCs. Five day old pups and 6-8 wks old adults were infected with RSV. The pDCs were purified using gradient density centrifugation and magnetic bead selection. The resulting cells from the purification were then labeled with CD11c and PDCA-1 antibodies to identify pDCs. (A) Purified pDCs from adult lung. (B) Purified pDCs from neonatal lung.

Neonatal RSV Infection Induced Limited Expression of *Irf-7*

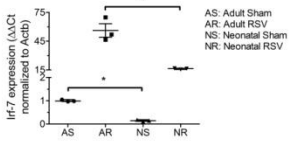


Fig 4: Relative expression of *Irf-7* in pulmonary pDCs. Five day old neonates or 6-8 wks old adults were infected with RSV. pDCs were purified at 1 day post infection; and the expression of *Irf-7* in these cells were quantified using real time PCR. NS: sham infected neonates; NR: RSV infected neonates; AS: sham infected adults; AR: RSV infected adults. *: $p<0.05$.

Neonatal RSV Infection Induced Limited Type I IFNs Response

	IFNα (ng/g lung protein)	IFNβ (ng/g lung protein)
NS	4.35 ± 0.78	5.57 ± 1.13
AS	3.77± 0.89	8.14 ± 2.31
NR	5.51± 1.02	11.8 ± 2.43*
AR	76.2 ± 11.2*#	42.3 ± 5.07*#

Fig 5: Type I IFNs in lung homogenates. Neonatal or adult mice were infected with RSV and total lung protein was isolated using T-Per (Pierce). IFNα and IFNβ were then measured using ELISA at 1 day post-infection. NS: sham infected neonates; NR: RSV infected neonates; AS: sham infected adults; AR: RSV infected adults. *: $p<0.05$; NR vs. NS or AR vs. AS; #: AR vs. NR.

Conclusions

- Neonatal pDCs express less *Irf-7* than adult pDCs at baseline.
- RSV infection induces *Irf-7* expression in both neonatal and adult pDCs; however, expression of *Irf-7* in pDCs from neonates is muted compared to adults.
- RSV infection induces limited amount of type I IFNs (IFNα and β) in neonates.
- The muted expression of *Irf-7* and resulting reduction in type I IFNs may play a role in neonatal RSV pathogenesis.

Acknowledgement

The project described was supported by LVC and Grant Number (5R01 AI090059) from NIAID. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIAID/NIH.

Example of a better poster

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

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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.

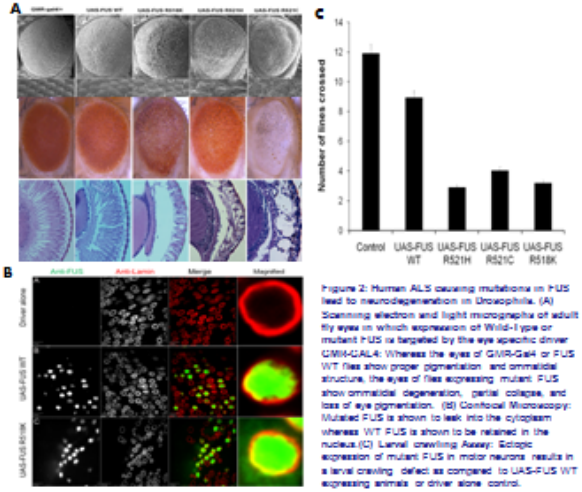


Figure 2: Human ALS causing mutations in FUS lead to neurodegeneration in Drosophila. (A) Scanning electron and light micrographs of adult fly eyes in which expression of Wild-type or mutant FUS is targeted by the eye specific driver GMR-GAL4: Whereas the eyes of GMR-Gal4 or FUS WT flies show proper 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.

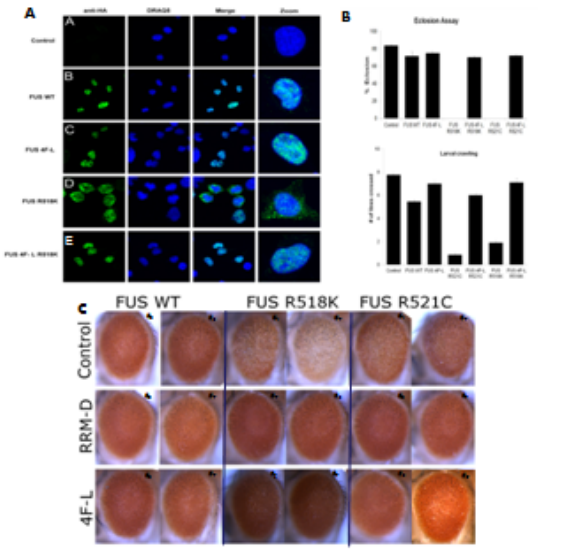


Figure 3: RNA-binding ability of FUS regulates toxicity and subcellular localization. (A) Confocal Imaging: In neuronal cells, WT FUS (B) is predominantly nuclear whereas FUS with ALS-linked mutation (D) is redistributed into the cytoplasm. RNA-binding incompetent FUS along with an ALS-linked mutation (E) is localized in the nucleus. (B) Behavioral Assays: When FUS was targeted by the motor-neuron specific driver (D42-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 Drosophila Eyes: 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.

Clinical research poster: get informed consent before using any patient photos

Health-Seeking Behavior and Primary Healthcare Needs in Rural Haiti

Carl Mickman, BS, Rex Suter, BS, Gretchen Newby, MPH, Alison Smith, BS, Charles Murphy, BS, Elizabethe Gleckler, PhD




BACKGROUND

- Since January 2010, Haiti has been the benefactor of increased foreign medical aid, yet health outcomes remain poor
- According to the WHO, Haiti suffers from the largest burden of disease, and is the poorest country per capita in the Americas
- Haitians living in rural areas, such as the Central Plateau, make up approximately 50% of the country's population, have worse health outcomes and are more impoverished than other Haitians
- Jacsonville, a community in the Central Plateau, has been receiving quarterly medical visits since 2009 from a Tulane University sponsored medical organization called Sante Total
- Previous research has shown that knowing baseline health needs and health-seeking behaviors in communities improves health outcomes long-term
- It has been shown that understanding health seeking behaviors and baseline health statistics can help direct resources in a more specific manner
- Primary health research into health issues in the Haitian Central Plateau is limited, and health-seeking behaviors are particularly understudied

OBJECTIVES

- To determine the financial, logistical, educational and cultural obstacles to healthcare in a small Haitian village served by a NGO medical clinic
- To determine the medical decision-making practices among villagers in using the clinic
- To determine measureable baseline health statistics in order to demonstrate future improvements in the clinic

METHODS

- We designed a multi-layered interview platform to examine and compare four cross-sectional samples of the community
- We used a two step approach to guide data collection
  1) Interviewed community leadership and local healthcare providers to guide second-tier interviews with local heads of household and teenagers (sexual health and education)
  2) Interview responses from community leadership and healthcare providers were compared to those of the heads of household and the teenagers
- Responses from first and second-tier interviews were used to guide a cross-sectional survey of 40 households, or 25% of the community
- Medical chart reviews were analyzed for disease incidence and compared to interviewee responses and surveys
- A survey of Sante Total clinic users was conducted and further assessed health seeking behaviors in the community
- A separate study analyzed hypertension prevalence in adults aged 35 and older in the community

RESULTS

Interview Results

Figure 1. Health Seeking Behavior among Haitians in Jacsonville Decision Tree

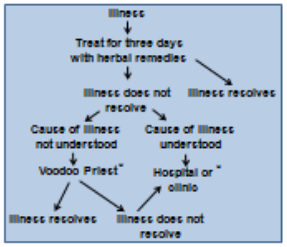
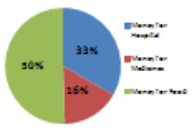


Figure 2. How heads of household would spend extra money to improve family health



Household Survey Results

Figure 3. N (%) of households reporting skipped/missed meals or not eating all day ≥ 1x in a 7 day period

Adults skip meals	97.5%
Adults don't eat all day	85.8%
Children skip meals	91.9%
Children don't eat all day	67.6%

Figure 4: Sites of Toilet Use

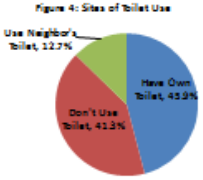


Chart Review Results

Figure 6. Symptom presentation in Sante Total pediatric consultations

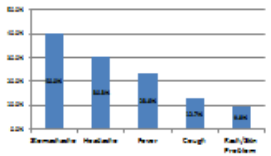


Figure 8. Percent of children categorized (WHO criteria) as stunted, severely stunted, underweight and severely underweight

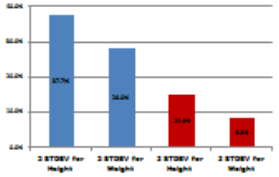
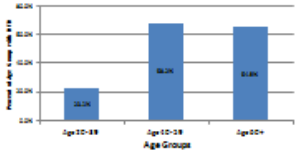
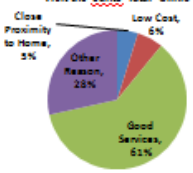


Figure 7. Prevalence of hypertension in different age groups



Clinic Survey Results

Figure 8: Why clinic users choose to visit the Sante Total Clinic



Major Themes Endorsed by Community Leadership, Healthcare Providers and Community Members

- Herbal remedies are universally used in the first three days of an illness, after which medical treatment is sought if possible
- Cost is the major deterent for villagers to seeking medical treatment
- Associated costs make medical appointment costs unpredictable as Haitians must pay extra for x-rays, ultrasounds, and other services
- Villagers choose healthcare providers based on reputation and would travel long distances to seek care, even bypassing closer facilities
- Voodoo practice is common in the community according to Community Leaders and Healthcare Providers, though no heads of household reported using Voodoo resources
- Voodoo Priests are a major provider of mental health care in the Haitian Central Plateau
- Voodoo Priests often refer patients to western medical services
- The teenage population has very low levels of education on sexual health topics

Community Survey Results

- More than half the community does not have access to toilet facilities
- Nearly all children are born at home in the village despite government programs providing free childbirth services
- Food availability decreases in the dry season but interviews suggest those that are part of a drip irrigation program have better crop results and thus more food

Clinic Survey Results

- The most important reason why people choose to come to the Sante Total clinic was that the services are good (not low cost or proximity to homes)

Chart Review Results

- Village children show high levels of both growth stunting and malnourishment, with teenage boys being those most at risk in both categories
- Preliminary studies show a large prevalence of hypertension with a disproportionate amount of adults with Stage 2 hypertension

DISCUSSION

- The presenting symptom triad of headache, stomachache and fever may point to continuing infection with intestinal worms in children, despite regular treatment with mebendazole
- Lack of sanitation infrastructure could be the cause of continued intestinal worm infection, and thus responsible for a large proportion of pediatric presentations as well as stunting and general malnutrition
- Health education with particular emphasis on basic sanitary practices could have farreaching effects on malnutrition and disease burden throughout the community
- Further studies could be used to monitor incidence of pediatric symptoms and to determine if implementation of sanitation infrastructure and education decreases burden of pediatric illness
- Further studies could be used to determine the effectiveness of drip irrigation programs by monitoring the height and weight of children in participating families