[1]Simmons University, Boston, MA
[2]Lousiana Cancer Research Center, New Orleans, LA
[3]Department of Cell and Molecular Biology, School of Science and Engineering, Tulane University, New Orleans, LA
[4]Department of Physiology, School of Medicine, Tulane University, New Orleans, LA

Introduction

- Angiogenesis is the process of new blood vessel formation from existing vessels. It is critical for embryonic development and wound repair, and it is essential for solid tumor progression and metastasis.
- We propose that endothelial cells are activated during angiogenesis to undergo partial endothelial-to-mesenchymal transition (pEndoMT) under control of master transcription factors Slug, Snail, Twist, Ovol1, and Ovol2.[1,2]

Hypothesis

Lentiviral constructs can drive constitutive overexpression of transcription factors that drive Endothelial-to-Mesenchymal Transition (pEndoMT) – e.g., Slug, Snail, Ovol1, and Ovol2 – in endothelial cells.

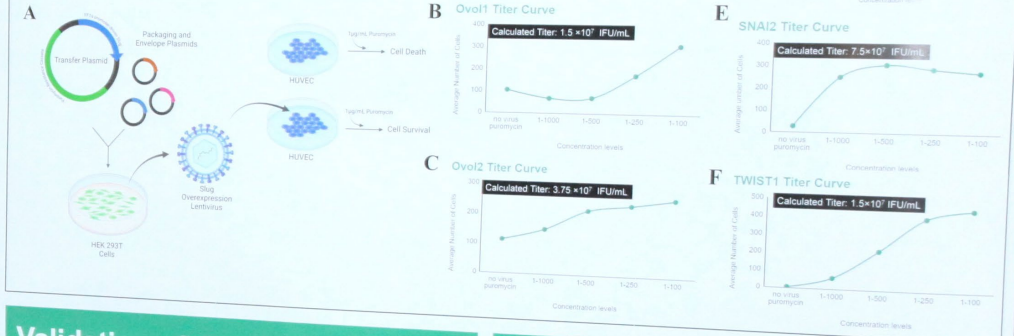
Overexpression of pEndoMT transcription factors will affect extent of sprouting angiogenesis.

Methods

- We generated Generation III lentivirus from published plasmids[3] to overexpress pEndoMT transcription genes in Human Umbilical Vein Endothelial Cells (HUVECS).
- Lentivirus was made in Human Embryonic Kidney (HEK) 293T packaging cells.
- Lentivirus was quantitatively titered in HUVEC by measuring dose-dependent puromycin resistance.
- Constitutive overexpression of pEndoMT transcription factors was confirmed in transduced HUVEC using quantitative PCR (qPCR).

Determination of Lentiviral Titer via Puromycin Resistance

Figure 1. Calculating Lentiviral Titer. A) Schematic of lentiviral production in 293T packaging cells, and transduction into HUVEC for quantification of viral titer by dose-dependent puromycin resistance. Figure generated in Biorender.com. Transduced HUVEC survival curves at 4 days following addition of puromycin were generated for overexpression lentiviruses for **B)** Ovol1, **C)** Ovol2, **D)** SNAI1 (Snail), **E)** SNAI2 (Slug), and **F)** TWIST1 (TWIST). Using these curves, we were able to calculate the concentration of infectious units (IFU) for each lentivirus.



Validation of Overexpression

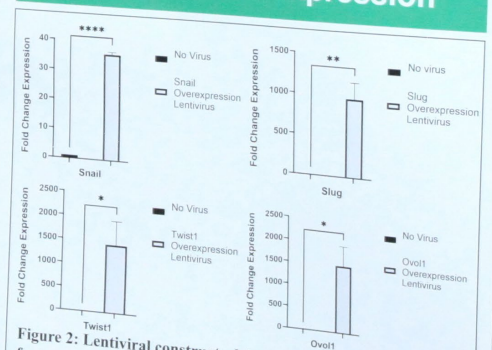
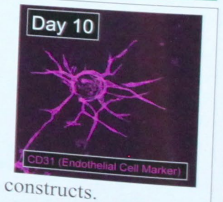


Figure 2: Lentiviral constructs drive constitutive transcription factor overexpression in HUVEC. HUVEC were transduced with lentiviral constructs (MOI = 1), and RNA was isolated after 4 days of transduction. Quantitative PCR (qPCR) was used to measure SNAI1 (Snail), SNAI2 (Slug), TWIST1 (Twist), and OVOL1 (Ovol1). Basal OVOL2 (Ovol2) expression was undetectable, thus a fold change could not be calculated for this gene.

Future Directions

Figure 3: Representative image of sprouting angiogenesis in Fibrin Gel Bead Assay. HUVEC cultured on collagen-coated beads sprout undergo angiogenesis in 3D fibrin hydrogel.



- Perform additional validation of our lentiviral overexpression constructs.
- Use Fibrin Gel Bead Assay to test if pEndoMT transcription factor overexpression in HUVEC alters sprouting angiogenesis.
- Use Tumor Angiogenesis-on-a-Chip platform to test if pEndoMT transcription factor overexpression in HUVEC alters tumor angiogenesis.

References

1. Fang, J. S., Hultgren, N. W., & Hughes, C. C. (2021). Regulation of partial and reversible endothelial-to-mesenchymal transition in angiogenesis. *Frontiers in Cell and Developmental Biology*, 9. doi:10.3389/fcell.2021.702021
2. Roca, H., Hernandez, J., Weidner, S., McEachin, R. C., Fuller, D., Sud, S., ... Pienta, K. J. (2013). Transcription factors OVOL1 and OVOL2 induce the mesenchymal to epithelial transition in human cancer. *PLoS ONE*, 8(10). doi:10.1371/journal.pone.0076773
3. Joung, J., Ma, S., Geiger-Schuller, K. R., Kirchgatterer, P. C., Verdine, V.K., ... Zhang, F. (2023). A transcription factor atlas of directed differentiation. *Cell*. 186(1): 209-229. doi: 10.1016/j.cell.2022.11.026.

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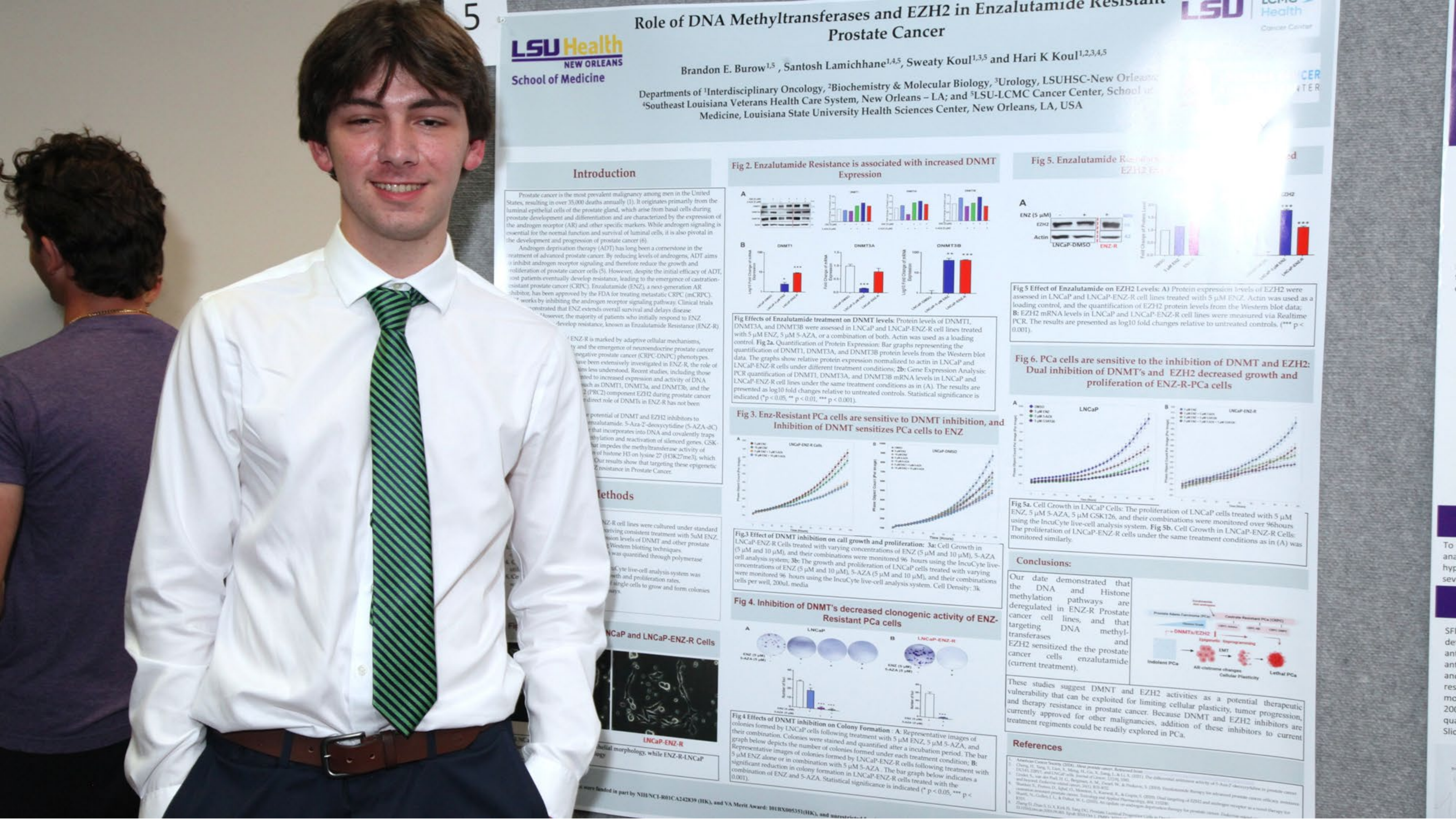

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Role of DNA Methyltransferases and EZH2 in Enzalutamide Resistant Prostate Cancer
LSU Health NEW ORLEANS
School of Medicine
Brandon E. Burow1,5 , Santosh Lamichhane1,4,5, Sweaty Koul1,3,5 and Hari K Koul1,2,3,4,5
Departments of 1Interdisciplinary Oncology, 2Biochemistry & Molecular Biology, 3Urology, LSUHSC-New Orleans
4Southeast Louisiana Veterans Health Care System, New Orleans – LA; and 5LSU-LCMC Cancer Center, School of
Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, USA
Introduction
Fig 2. Enzalutamide Resistance is associated with increased DNMT Expression
Fig Effects of Enzalutamide treatment on DNMT levels: Protein levels of DNMT1, DNMT3A, and DNMT3B were assessed in LNCaP and LNCaP-ENZ-R cell lines treated with 5 μM ENZ, 5 μM 5-AZA, or a combination of both. Actin was used as a loading control. Fig 2a. Quantification of Protein Expression: Bar graphs representing the quantification of DNMT1, DNMT3A, and DNMT3B protein levels from the Western blot data. The graphs show relative protein expression normalized to actin in LNCaP and LNCaP-ENZ-R cells under different treatment conditions; 2b: Gene Expression Analysis: PCR quantification of DNMT1, DNMT3A, and DNMT3B mRNA levels in LNCaP and LNCaP-ENZ-R cell lines under the same treatment conditions as in (A). The results are presented as log10 fold changes relative to untreated controls. Statistical significance is indicated (*p < 0.05, ** p < 0.01, *** p < 0.001).
Fig 3. Enz-Resistant PCa cells are sensitive to DNMT inhibition, and Inhibition of DNMT sensitizes PCa cells to ENZ
Fig 4. Inhibition of DNMT's decreased clonogenic activity of ENZ-Resistant PCa cells
Fig 5. Enzalutamide
Fig 5 Effect of Enzalutamide on EZH2 Levels: A) Protein expression levels of EZH2 were assessed in LNCaP and LNCaP-ENZ-R cell lines treated with 5 μM ENZ. Actin was used as a loading control, and the quantification of EZH2 protein levels from the Western blot data; B: EZH2 mRNA levels in LNCaP and LNCaP-ENZ-R cell lines were measured via Realtime PCR. The results are presented as log10 fold changes relative to untreated controls. (*** p < 0.001).
Fig 6. PCa cells are sensitive to the inhibition of DNMT and EZH2: Dual inhibition of DNMT's and EZH2 decreased growth and proliferation of ENZ-R-PCa cells
Fig 5a. Cell Growth in LNCaP Cells: The proliferation of LNCaP cells treated with 5 μM ENZ, 5 μM 5-AZA, 5 μM GSK126, and their combinations were monitored over 96hours using the IncuCyte live-cell analysis system. Fig 5b. Cell Growth in LNCaP-ENZ-R Cells: The proliferation of LNCaP-ENZ-R cells under the same treatment conditions as in (A) was monitored similarly.
Conclusions:
Our date demonstrated that the DNA and Histone methylation pathways are deregulated in ENZ-R Prostate cancer cell lines, and that targeting DNA methyl-transferases and EZH2 sensitized the the prostate cancer cells enzalutamide (current treatment).
These studies suggest DMNT and EZH2 activities as a potential therapeutic vulnerability that can be exploited for limiting cellular plasticity, tumor progression, and therapy resistance in prostate cancer. Because DNMT and EZH2 inhibitors are currently approved for other malignancies, addition of these inhibitors to current treatment regiments could be readily explored in PCa.
References
LNCaP and LNCaP-ENZ-R Cells
LNCaP-ENZ-R

Molly Chaffee [1], Kayla Vo [2], Ryan J. Schroeder [2], Luis Marrero [3]
Tulane University [1], Louisiana State University Health Sciences Center School of Medicine [2], Department of Orthopaedics [3]

School of Medicine
Department of Orthopaedics

Introduction

Synovial fibrosis (SFb), a painful contracture limiting joint motion and quality of life, is a hallmark of arthrofibrosis (AF), a common complication after joint repair. SFb is categorized by low (<41%), moderate (42-54%), and high (>54%) collagen deposition levels and is a significant challenge in osteoarthritis (OA) patients. As shown in Figure 1, transforming growth factor beta 1 (TGFβ1) drives SFb and regulates essential cell processes. Interleukin (IL) 11, synthesized downstream of the TGFβ1-mediated JAK/STAT3 cascade, promotes fibrosis if dysregulated. Novomedix's (NMX) novel inhibitors selectively target IL11 without disrupting TGFβ1-mediated functions. These inhibitors effectively reduce IL11-driven collagen deposition in OA-derived fibrotic synoviocytes.

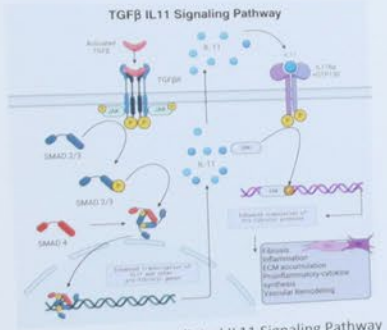


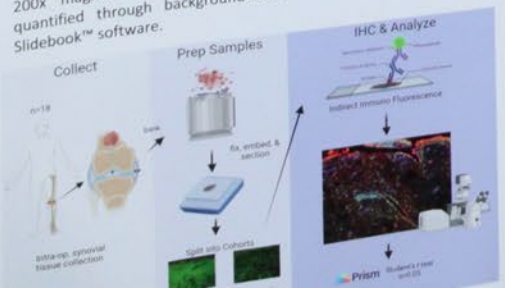
Figure 1: The TGFb1 mediated IL11 Signaling Pathway

Objective and Significance

To assess the potential of NMX for in vivo SFb treatment, this study analyzes IL11 co-expression with TGFβ1 in banked knee OA samples, hypothesizing a correlation between IL11 expression and SFb severity.

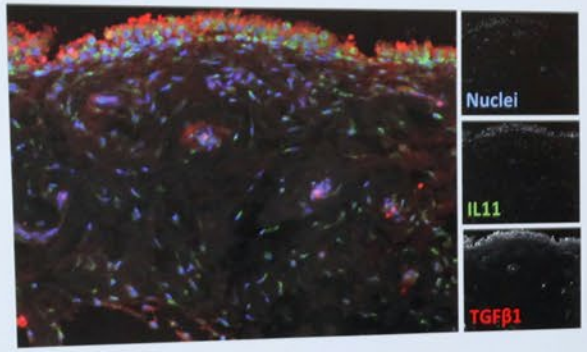
Methods

SFb cohorts were based on pre-defined histological scores. Co-detection of TGFβ1 and IL11 by indirect immunofluorescence used anti-TGFβ1 (mouse monoclonal) and anti-IL11 (rabbit polyclonal) antibodies. Sections were then stained with anti-mouse Alexa 594 and anti-rabbit Alexa 647 secondary antibodies for TGFβ1 and IL11, respectively, along with DAPI nuclear counterstain. Samples were mounted and imaged using a confocal microscope (Olympus) at 200x magnification. Co-expression of TGFβ1 and IL11 was quantified through background-corrected signal analysis using Slidebook™ software.



Results

Low Fibrosis



High Fibrosis

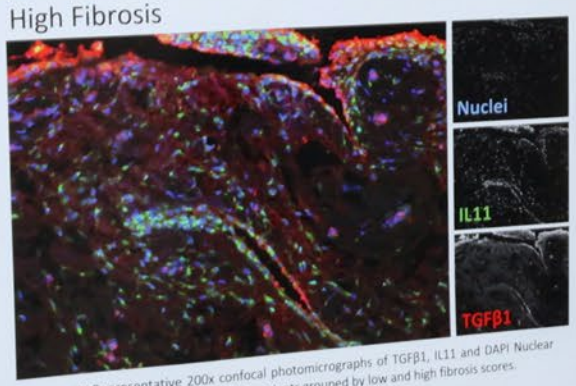


Figure 2: Representative 200x confocal photomicrographs of TGFβ1, IL11 and DAPI Nuclear counterstain in the synovium of kOA patients grouped by low and high fibrosis scores.

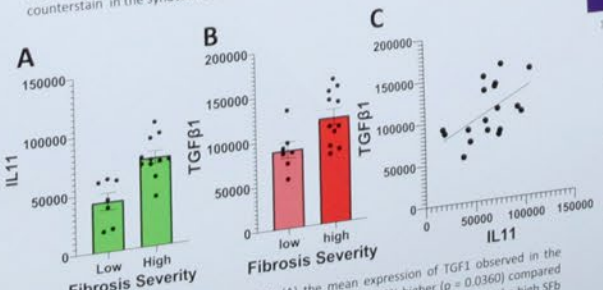


Figure 3: Student T test calculated that (A) the mean expression of TGF1 observed in the synovium of patients classified with high SFb severity was 35% higher ($p = 0.0360$) compared to the signal measured from the low severity SFb group and (B) IL11 expression in the high SFb severity were registered at a 77% increase ($p = 0.0016$) from patients with less severe SFb. Pearson's correlation revealed (C) a moderate but significant correlation between TGFβ1 and IL11 ($R = 0.51$; $p = 0.0314$).

Discussion and Limitations

- Increased expression of IL11 relates to TGFβ1 in agreement with SFb severity. While this study does not prove causality, it suggests a relationship between IL11 and SFb, highlighting the diseased synovium as an effective target for NMX administration.
- The study is limited by sample size and doesn't account for confounding variables such as synovitis grade and presence of additional pro-fibrotic factors such as connective tissue growth factor.
- Further studies will investigate the effectiveness of NMX on aberrant collagen deposition, contraction, and myofibroblast differentiation rate of patient-derived synovial fibroblasts.

Conclusion

- IL11 levels in patient synovial tissue correlate to TGFβ1 levels and severity of SFb. While this study does not prove causality, it provides further evidence that IL 11 and SFb are interrelated.
- Further studies will investigate the effectiveness of the NMX compound in patient synovial tissue.
- This study indicates the potential supplementation of NMX to assist manipulation under anesthesia and arthroscopic lysis of adhesions in the management of debilitating arthrofibrosis.

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References

1. Blaney Davidson EN, van der Kraan PM, van den
beta and osteoarthritis. Osteoarthritis
2007;15(6):597-604. doi:10.1016/j.joca.2007.02.

1. Schafer S, Viswanathan S, Widjaja AA, et al. IL-
determinant of cardiovascular fibros
2017;552(7683):110-115. doi:10.1038/nature2467

1. Ng et al. Interleukin-11 signaling underli
parenchymal dysfunction, and chronic inflamma
airway. Experimental & Molecular Medicine (2020
1878; 15

1. Geng et al. PD-L1 on invasive fibroblasts drives fibr
humanized model of idiopathic pulmonary fibrosis. JC
2019

1. Hodgeson S, O'Brien S, Simkin J, Plakotaris E, McCarthy C
V, Marrero L. Differences in synovial fibrosis relative to ran
motion in knee osteoarthritis patients. J Orthop Res. 2
Mar;40(3):584-594. doi: 10.1002/jor.25061. Epub 2021 May
PMID: 33913554; PMCID: PMC8553814.

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LSU Health NEW ORLEANS School of Medicine

[1]Jack C. Erickson-King, [1]Jack R. Elliott, [1]Megan C. Benz, [1]Thomas Cheng, [1]Della A... Carlino, [1]Bridgette M. Collins-Burow, [2]Bolin Liu, [1]Van T. Hoang, [1]Elizabeth C. Martin, [1]Stephen M. Boué, [1]Matthew E. Burow

[1]Tulane University School of Medicine | Tulane Cancer Center

[2]Louisiana State University School of Medicine, Department of Genetics

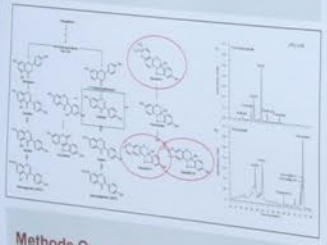
Abstract

For women worldwide, breast cancer (BC) ranks top 2 in both annual quantity of cases and deaths among cancers. Leading these statistics, developing new methods for combating breast cancer remains at the forefront of cancer research. Types of breast cancer are differentiated based on the presence of 3 receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). HER2+ BC targeted agents such as trastuzumab, pertuzumab, and lapatinib, alone or in combination with traditional chemotherapeutics, have become the standard of care for this BC subtype, significantly improving patient survival rates. However, the development of resistance to targeted therapy represents a major obstacle in the treatment of HER2+ breast cancer, highlighting a critical need to identify novel therapeutic targets to treat resistant HER2+ breast cancer.

Isoflavonoids, an important class of natural compounds produced from numerous plant sources including legumes, have been identified to be beneficial for human health. A member of the legume family, the soybean, maintains a high isoflavonoid content, specifically daidzein and stress induced derivatives called glyceollins. Recent studies have demonstrated glyceollin activity against ER+ breast cancer due to inhibition of ER and its associated pathways. To date the impact of glyceollins on other breast cancer subtypes has not been fully explored. Here, we utilized a panel of HER2+ breast cancer cell lines (HCC 1954, Au565, SKBR3), as well as derived trastuzumab-resistant variants (herceptin-resistant SKBR3), to evaluate the effects of glyceollin treatment, alone or in combination with other targeted agents, on cell viability and clonogenicity. Additionally, we analyzed changes in downstream gene expression using qRT-PCR profiler array for Human Cancer Pathways to define potential glyceollin-targeted pathways involved in the regulation of HER2 breast cancer cell biology.

Results demonstrated that glyceollin decreased cell survival and colony formation across cell lines. Additionally, glyceollin suppressed expression of pro-oncogenic genes in herceptin-resistant SKBR3 cells. Our preliminary findings provide support for a novel approach using isoflavonoids in the development of targeted therapy for HER2+ BC.

Glyceollin Biosynthesis



Methods Overview

Reduction of Cell Viability

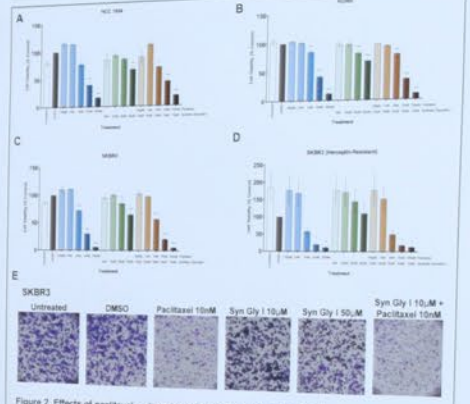


Figure 2. Effects of paclitaxel and synthetic glyceollin I alone or combination treatment on viability of HER2+ cell lines. HER2+ cell lines **(A)** HCC 1954, **(B)** AU565 mCherry, SKBR3 **(C)** parental and **(D)** herceptin-resistant were plated on 96-well costar flat-bottom plates (6K cells per well for all cell lines, except AU565 at 10K cells per well). Cells were treated with vehicle (DMSO), paclitaxel, and synthetic glyceollin I (Syn Gly I) alone or in combination. On day 3 post treatment, plates were fixed and stained with crystal violet **(E** (SKBR3)). Plates were imaged for cell morphology analysis using the BioTek Cytation 5 Imaging Reader (CY). Plates were eluted with acetic acid then read at 590nm on the CY. Data shown represent mean ± SEM, n=3. * p-value < 0.05; ** p < 0.01; *** p < 0.001.

Effects on colony formation

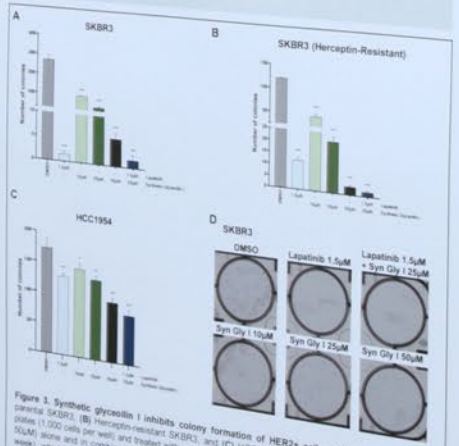


Figure 3. Synthetic glyceollin I inhibits colony formation of HER2+ cells. HER2+ cells lines **(A)** parental SKBR3, **(B)** Herceptin-resistant SKBR3, and **(C)** HCC 1954 were plated on 6-well flat bottom plates (1,000 cells per well) and treated with vehicle, lapatinib, or synthetic glyceollin I (10μM, 25μM, and 50μM) alone and in combination. Cells were cultured until colonies of 30-50 cells formed (at least one week), which varied between cell lines. Cells were fixed with glutaraldehyde then stained with crystal violet and imaged using the CY. Graphs were generated with GraphPad Prism software. Data shown represent average number of colonies ± SEM, n=3. * p-value < 0.05; ** p < 0.01; *** p < 0.001.

Differential Gene Expression

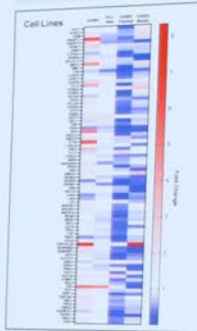


Figure 4. Cancer pathway analysis of cells treated with synthetic glyceollin I. HER2+ cell lines HCC 1954, Au565 mCherry, parental SKBR3, and Herceptin-resistant SKBR3 were treated with synthetic glyceollin I (25μM) for 24 hours. RNA was extracted from cell pellets using the Zymo Research Quick-RNA MiniPrep Kit. RNA Extraction Kit. Concentration and purity of RNA samples were evaluated using a Nanodrop, and cDNA was synthesized via the Qiagen RT First Strand Kit cDNA Synthesis Kit. Gene expression was assessed using the Qiagen Human Cancer Pathway Finder PCR Array Plate. PCR array plates were run on the CFX Opus 96 RT-PCR System, and heat maps were generated using GraphPad Prism software.

Conclusions and Future Directions

Conclusions

- Synthetic glyceollin I treatment significantly inhibits proliferation and viability of HER2+ breast cancer cells in a dose-dependent manner.
- Expression of cancer-related genes was altered in HCC1954 cells treated with Syn Gly I, notably TEK which is involved in angiogenesis.
- The majority of genes in the cancer pathway PCR array were downregulated in Syn Gly I-treated herceptin-resistant SKBR3 cells compared to herceptin-responsive cells, suggesting enhanced sensitivity of the former to Syn Gly I treatment.

Future Directions

- Assess effects of Syn Gly I treatment on cell viability and gene expression of additional herceptin-responsive and -resistant HER2+ cell lines.
- Perform qRT-PCR to validate PCR array gene targets.

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References

1. Butti, R., Das, S., Gunasekaran, V. P., Yadav, A. S., Kumar, D., & Kundu, G. C. (2018). Receptor tyrosine kinases (RTKs) in breast cancer: signaling, therapeutic implications and challenges. *Molecular cancer*, *17*(1), 34.
2. Hsu, J.L., Hung, MC. The role of HER2, EGFR, and other receptor tyrosine kinases in breast cancer. *Cancer Metastasis Rev* **35**, 575–588 (2016).
3. Nahta, R., Esteva, F.J. HER2 therapy: Molecular mechanisms of trastuzumab resistance. *Breast Cancer Res* **8**, 215 (2006).
4. Zhiyong Yue, Shanhong He, Jinpei Wang, Qi Jiang, Hanping Wang, Jia Wu, Chenxi Li, Zixian Wang, Xuan He, Nannan Jia. Glyceollins from soybean: Their pharmacological effects and biosynthetic pathways. *Heliyon* **9**, 11 (2023).

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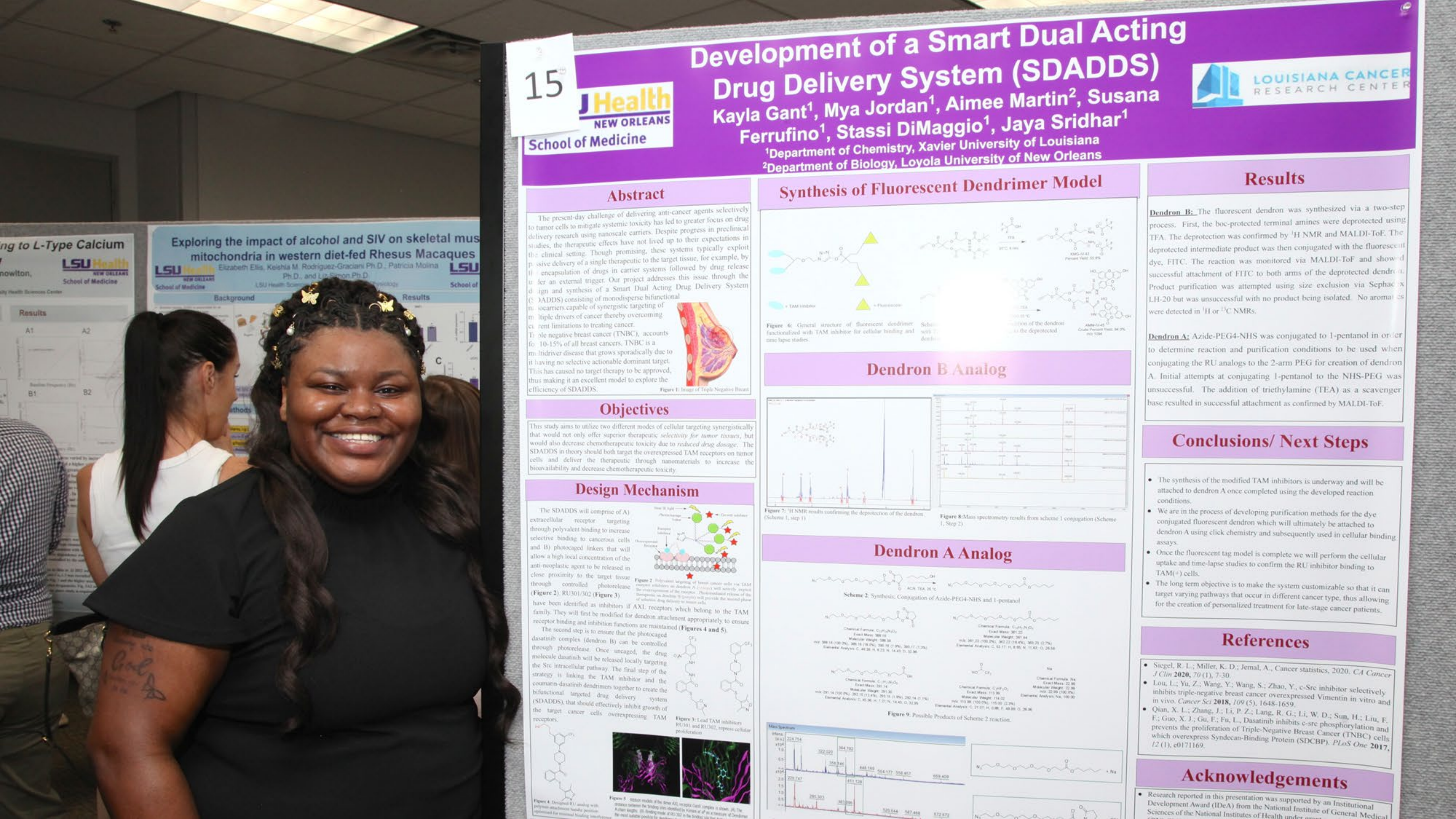
Tulane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Development of a Smart Dual Acting Drug Delivery System (SDADDS)
Kayla Gant1, Mya Jordan1, Aimee Martin2, Susana Ferrufino1, Stassi DiMaggio1, Jaya Sridhar1
1Department of Chemistry, Xavier University of Louisiana
2Department of Biology, Loyola University of New Orleans
Health NEW ORLEANS School of Medicine
LOUISIANA CANCER RESEARCH CENTER
Abstract
Objectives
This study aims to utilize two different modes of cellular targeting synergistically that would not only offer superior therapeutic selectivity for tumor tissues, but would also decrease chemotherapeutic toxicity due to reduced drug dosage. The SDADDS in theory should both target the overexpressed TAM receptors on tumor cells and deliver the therapeutic through nanomaterials to increase the bioavailability and decrease chemotherapeutic toxicity.
Design Mechanism
Synthesis of Fluorescent Dendrimer Model
Dendron B Analog
Dendron A Analog
Scheme 2: Synthesis; Conjugation of Azide-PEG4-NHS and 1-pentanol
Figure 9. Possible Products of Scheme 2 reaction.
Results
Dendron B: The fluorescent dendron was synthesized via a two-step process. First, the boc-protected terminal amines were deprotected using TFA. The deprotection was confirmed by 1H NMR and MALDI-ToF. The deprotected intermediate product was then conjugated with the fluorescent dye, FITC. The reaction was monitored via MALDI-ToF and showed successful attachment of FITC to both arms of the deprotected dendron. Product purification was attempted using size exclusion via Sephadex LH-20 but was unsuccessful with no product being isolated. No aromatics were detected in 1H or 13C NMRs.
Dendron A: Azide-PEG4-NHS was conjugated to 1-pentanol in order to determine reaction and purification conditions to be used when conjugating the RU analogs to the 2-arm PEG for creation of dendron A. Initial attempts at conjugating 1-pentanol to the NHS-PEG was unsuccessful. The addition of triethylamine (TEA) as a scavenger base resulted in successful attachment as confirmed by MALDI-ToF.
Conclusions/ Next Steps
The synthesis of the modified TAM inhibitors is underway and will be attached to dendron A once completed using the developed reaction conditions.
We are in the process of developing purification methods for the dye conjugated fluorescent dendron which will ultimately be attached to dendron A using click chemistry and subsequently used in cellular binding assays.
Once the fluorescent tag model is complete we will perform the cellular uptake and time-lapse studies to confirm the RU inhibitor binding to TAM(+) cells.
The long term objective is to make the system customizable so that it can target varying pathways that occur in different cancer type, thus allowing for the creation of personalized treatment for late-stage cancer patients.
References
Acknowledgements
Exploring the impact of alcohol and SIV on skeletal muscle mitochondria in western diet-fed Rhesus Macaques

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Exploring the Mechanisms of Anticancer Agents with Improved Solubility Against Triple Negative Breast Cancer

LSU Health NEW ORLEANS School of Medicine

LOUISIANA CANCER RESEARCH CENTER

Paige Goderis, Gabrielle Vontz, Zhipin Liang, Connor Kent, Lei Liu, Caiyue Li and Qiang Shen

Department of Interdisciplinary Oncology, Louisiana State University Health Sciences Center, LSU-LCMC Health Cancer Center, New Orleans, LA.

Introduction

Triple-negative breast cancer (TNBC) poses significant challenges in treatment due to its aggressive nature, lack of targeted therapy options, and high resistance rates. TNBC's metabolic dependency on aberrant glycolytic pathways, termed the Warburg effect, provides a potential avenue to develop novel targeted therapies. The Shen Lab investigates mitochondrial and metabolic targets for TNBC to develop innovative anticancer small molecules from synthetic compounds and natural products. Our lab has previously developed a highly soluble derivative of natural product oridonin, CYD0618, that has increased potency against breast cancer in both cell culture experiments and xenograft mouse models. In parallel studies, the lab developed HJC0152, a derivative of FDA-approved anthelmintic niclosamide, that exhibits improved solubility and bioavailability. Despite improvements in potency and bioavailability, specific mechanisms of action of CYD0618 and HJC0152 against breast cancer are understudied. In this project, we evaluated the effects of CYD0618 and HJC0152 on TNBC viability and metabolism using MTT assays, ADP/ATP ratio measurements and Seahorse analysis. Results from this project will contribute to understanding the mechanisms of promising anticancer agents and provide a foundation to optimize these compounds for future clinical testing.

CYD0618

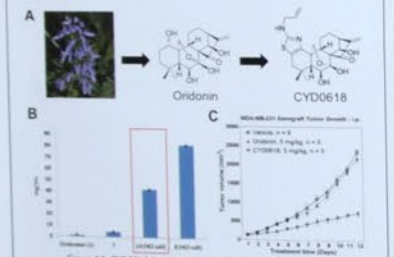


Figure 1A: CYD0618 is a derivative of natural product oridonin.

Figure 1B: CYD0618 exhibits increased solubility compared to parent compound oridonin.

Figure 1C: CYD0618 inhibits tumor growth in a xenograft TNBC mouse model.

HJC0152

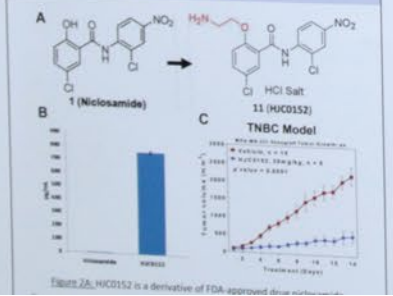
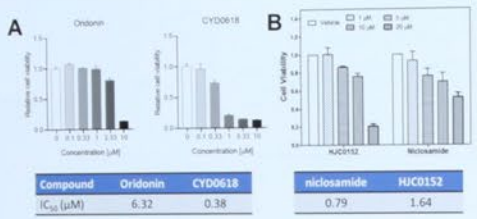


Figure 2A: HJC0152 is a derivative of FDA-approved drug niclosamide

MTT Assays



Compound	Oridonin	CYD0618
IC_{50} (μM)	6.32	0.38

niclosamide	HJC0152
0.79	1.64

Figure 3A: CYD0618 shows improved potency against TNBC cells *in vitro* compared to oridonin.

Figure 3B: HJC0152 exhibits comparable potency against TNBC to niclosamide.

ADP/ATP Ratio

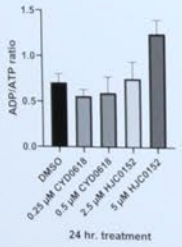


Figure 4: HJC0152 treatment increases the ADP/ATP ratio in TNBC cells in a dose dependent manner, while CYD0618 does not significantly impact the ADP/ATP ratio.

Seahorse Analysis

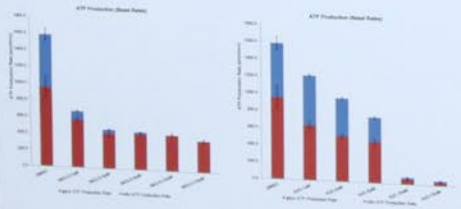


Figure 5: While niclosamide largely targets mitochondrial ATP production,

Conclusions

- CYD0618 is derivative of oridonin that exhibits improved solubility and potency against TNBC.
- HJC0152 is a derivative of niclosamide that exhibits improved solubility and bioavailability.
- Both CYD0618 and HJC0152 inhibit TNBC growth in vitro.
- HJC0152 inhibits ATP production through both mitochondrial phosphorylation and glycolysis.
- CYD0618 and HJC0152 are promising anticancer agents for further investigation for clinical applications.

Future Direction

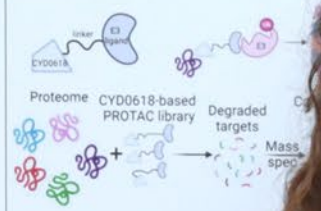


Figure 6: Degradation based protein profiling (DBPP) identify direct protein targets of sp

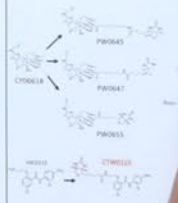


Figure 7: Biotin-labeled tool co

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How Environmental Risk Factors Drive Racial Disparities in Prostate Cancer Stage Diagnosis

Randy Hamilton[1], Nubaira Rizvi, M.S[2], Xiaowen Yang[2], Michael Celestin Jr., PhD[2], Ty-Runet Bryant, MPH[2], Almetra Granger, MPH[2], Tung Sung Tseng, DrPH[2], Xiao-Cheng Wu, MD[2], Qingzhao Yu, PhD[2]

Xavier University of Louisiana[1], LSU Health Sciences New Orleans[2]

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Introduction

- Prostate cancer is the leading cause of cancer among men in the U.S, primarily aged 65 and older.
- In research, prostate cancer shows significant, disproportional effects of 73% higher incidence rates and more than double higher rates of mortality in African American men when compared to Caucasian men.
- Additionally, AA men are diagnosed in younger ages with more aggressive tumors and advanced stages.
- Studies have linked these disparities to be associated with risk factors of socioeconomic, environmental, or biological influencers.
- In our study, we hypothesized environmental factors to be a contributor to the racial disparities in prostate cancer stage at diagnosis between African American and Caucasian men.

Objective

- Identify environmental variables associated with racial disparities in prostate cancer diagnosis outcomes among African American and Caucasian men and quantify their risk.

Methodology

- **Data Source**: Louisiana Tumor Registry (LTR) data from 2010 to 2018, 2010 Census Tract data, and Environmental Justice Index (EJI) data.
- **Participants**: N=24,647 men in Louisiana; African American (n=8,772 [36%]) and Caucasian (n=15,875 [64%])
- **Variables**: Outcome – stage at diagnosis, exposure – African American vs Caucasian, Exploratory Variables - (Marital Status, Insurance, Poverty, CDI, Ozone, Comorbidity, Acetaldehyde, Coal, Cancer Risk, etc.)
- **Methods**: Chi-Square test of association, T-Test, ANOVA, and Multiple Mediation Analysis were used to determine our variables' connection to racial disparity in stage
- **Analysis**: R Studio

Mediator chart of association between predictor, indicator,

Results

	Chi-Square	P-Value
Race	10.406	0.001

*Test of association between stage and race

	Black/African American (N=8772)	White/ Caucasian (N=15875)	P-value
Stage			0.00126
Early stage	6905 (78.7%)	12771 (80.4%)	
Late stage	1867 (21.3%)	3104 (19.6%)	
Marital Status			<0.001
Married	4593 (52.4%)	10822 (68.2%)	
Not Married	3348 (38.2%)	3291 (20.7%)	
Insurance			<0.001
Private Insurance	2989 (34.1%)	6609 (41.6%)	
Public Insurance	5416 (61.7%)	8472 (53.4%)	
BMI			0.00523
Mean (SD)	29.2 (6.25)	29.4 (5.44)	
Comorbidity			<0.001
0	6765 (77.1%)	13457 (84.8%)	
1 or 2	1736 (19.8%)	2204 (13.9%)	
3 or above	271 (3.1%)	214 (1.3%)	
Poverty Indicator			<0.001
Mean (SD)	3.49 (0.749)	2.83 (0.917)	
Acetaldehyde			<0.001
Mean (SD)	2920 (14900)	2050 (12500)	
Cancer Risk due to Air Toxics			<0.001
Mean (SD)	0.964 (0.120)	0.858 (0.136)	
% of housing built before 1980 (Lead Exposure)			<0.001
Mean (SD)	59.9 (21.8)	46.7 (22.9)	
Neighborhood Walkability			<0.001
Mean (SD)	7.74 (3.21)	6.75 (2.81)	
Proximity to Railroad (1 mile radius)			<0.001
Mean (SD)	56.6 (37.1)	33.6 (33.4)	
Concentration of Asthma in the Community			<0.001
Mean (SD)	10.7 (1.47)	9.26 (1.13)	

Table 1: Comparison of socioeconomic and environmental variables between Black and White patients with p-value

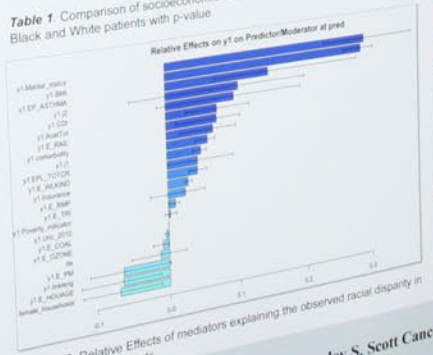


Figure 2: Relative Effects of mediators explaining the observed racial disparity in Prostate cancer patients.

Results (Cont.)

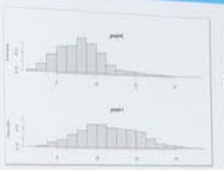


Figure 3: Pred=0 is White and Pred=1 is Black. Higher proportion of White patients lived in areas where proportion of population with Asthma is low compared to Black patients.

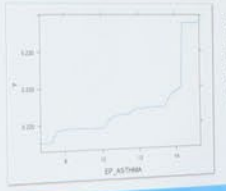


Figure 4: Living in areas with increased proportion of Asthma patients increases the probability of getting diagnosed at a later stage.

Discussion

- Our investigation found environmental variables to have a partial contribution in explaining the observed racial disparities in prostate cancer stage diagnosis through potential associations in Acetaldehyde (7.1%) and Cancer Risk due to Air Toxics (7.3%)
- Findings in this study were however consistent with previous literature showing that SES factors such as BMI (35.9%), marital status (31.9%), and insurance (7.3%) provide more significant impact in the disparity of prostate cancer diagnosis when compared to environmental exposure.
- Limitations: No genetic data were collected for the analysis, variables were limited to cancer registry standard data, and a study of African American and Caucasian cancer patients were only able to be considered due the sample size that was too small for other races.

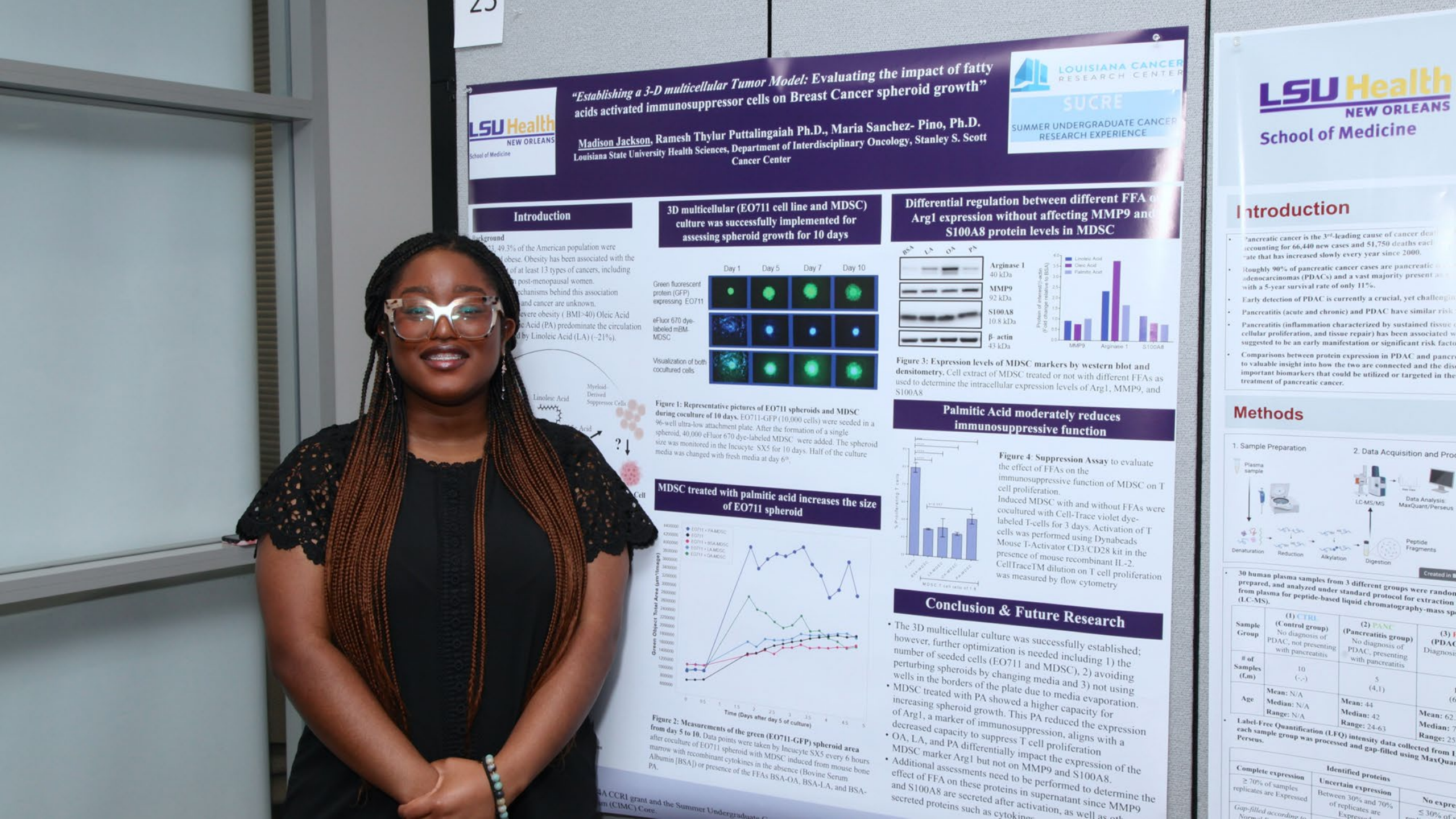
Conclusion

- Environmental risk factors including Acetaldehyde, Concentration of Asthma in the Community, and Cancer Risk due to Air Toxins were identified in contributing an association among racial disparities in prostate cancer stage diagnosis between African American and Caucasian men.
- Further studies should investigate what chemical substances lead to air toxicity and cancer development in neighborhoods to help indicate other associate factors of disparities within cancer diagnosis.

Acknowledgments

•The Tobacco Control Initiative and Louisiana Tumor Registry

This research project was supported by the Stanley S. Scott Cancer Center.


"Establishing a 3-D multicellular Tumor Model: Evaluating the impact of fatty acids activated immunosuppressor cells on Breast Cancer spheroid growth"
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SUMMER UNDERGRADUATE CANCER RESEARCH EXPERIENCE
Madison Jackson, Ramesh Thylur Puttalingaiah Ph.D., Maria Sanchez- Pino, Ph.D.
Louisiana State University Health Sciences, Department of Interdisciplinary Oncology, Stanley S. Scott Cancer Center
Introduction
3D multicellular (EO711 cell line and MDSC) culture was successfully implemented for assessing spheroid growth for 10 days
Differential regulation between different FFA
Arg1 expression without affecting MMP9 and
S100A8 protein levels in MDSC
Day 1
Day 5
Day 7
Day 10
Green fluorescent protein (GFP) expressing EO711
eFluor 670 dye-labeled mBM-MDSC
Visualization of both cocultured cells
Figure 1: Representative pictures of EO711 spheroids and MDSC during coculture of 10 days.
MDSC treated with palmitic acid increases the size of EO711 spheroid
Time (Days after day 5 of culture)
Figure 2: Measurements of the green (EO711-GFP) spheroid area from day 5 to 10.
BSA
LA
OA
PA
Arginase 1
40 kDa
MMP9
92 kDa
S100A8
10.8 kDa
β- actin
43 kDa
Linoleic Acid
Oleic Acid
Palmitic Acid
MMP9
Arginase 1
S100A8
Figure 3: Expression levels of MDSC markers by western blot and densitometry.
Palmitic Acid moderately reduces immunosuppressive function
Figure 4: Suppression Assay
Conclusion & Future Research
The 3D multicellular culture was successfully established; however, further optimization is needed including 1) the number of seeded cells (EO711 and MDSC), 2) avoiding perturbing spheroids by changing media and 3) not using wells in the borders of the plate due to media evaporation.
MDSC treated with PA showed a higher capacity for increasing spheroid growth. This PA reduced the expression of Arg1, a marker of immunosuppression, aligns with a decreased capacity to suppress T cell proliferation
OA, LA, and PA differentially impact the expression of the MDSC marker Arg1 but not on MMP9 and S100A8.
Additional assessments need to be performed to determine the effect of FFA on these proteins in supernatant since MMP9 and S100A8 are secreted after activation, as well as other secreted proteins such as cytokines
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Introduction
Methods
1. Sample Preparation
2. Data Acquisition and Proc
Plasma sample
LC-MS/MS
Data Analysis: MaxQuant/Perseus
Denaturation
Reduction
Alkylation
Digestion
Peptide Fragments

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"Establishing a 3-D multicellular Tumor Model: Evaluating the impact of fatty acids activated immunosuppressor cells on Breast Cancer spheroid growth"

Madison Jackson, Ramesh Thylur Puttalingaiah Ph.D., Maria Sanchez- Pino, Ph.D.
Louisiana State University Health Sciences, Department of Interdisciplinary Oncology, Stanley S. Scott Cancer Center

LOUISIANA CANCER RESEARCH CENTER
SUCRE
SUMMER UNDERGRADUATE CANCER RESEARCH EXPERIENCE

Introduction

3D multicellular (EO711 cell line and MDSC) culture was successfully implemented for assessing spheroid growth for 10 days

Differential regulation between different FFA on Arg1 expression without affecting MMP9 and [illegible] levels in MDSC

Methods

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Proteomics Analysis of PDAC and Pancreatitis Plasma Samples: Investigating Associations and Differences

Amelia Jiang-Yu[1], Amirsalar Mansouri[2,3], Marek Benes[4], Mary Maluccio[2,3], Nicholas J. Skill[2,3], Jiri Adamec[2,3]

[1]Johns Hopkins University, Baltimore, MD, United States
[2]Louisiana Cancer Research Center, New Orleans, LA, United States
[3]Louisiana State University – Health Sciences Center, New Orleans, LA, United States
[4]Charles University, 3rd Faculty of Medicine, Prague, Czech Republic

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Introduction

- Pancreatic cancer is the 3rd leading cause of cancer death in the U.S., accounting for 66,440 new cases and 51,750 deaths each year – a mortality rate that has increased slowly every year since 2000.
- Roughly 90% of pancreatic cancer cases are pancreatic ductal adenocarcinomas (PDACs) and a vast majority present as advanced-stage with a 5-year survival rate of only 11%.
- Early detection of PDAC is currently a crucial, yet challenging, goal.
- Pancreatitis (acute and chronic) and PDAC have similar risk factors.
- Pancreatitis (inflammation characterized by sustained tissue damage, cellular proliferation, and tissue repair) has been associated with and is suggested to be an early manifestation or significant risk factor of PDAC.
- Comparisons between protein expression in PDAC and pancreatitis may lead to valuable insight into how the two are connected and the discovery of important biomarkers that could be utilized or targeted in the diagnosis and treatment of pancreatic cancer

Methods

1. Sample Preparation
2. Data Acquisition and Processing

Human plasma samples from 3 different groups were randomized, aliquoted, and analyzed under standard protocol for extraction of proteins from plasma for peptide-based liquid chromatography-mass spectroscopy (LC-MS).

	(1) CTRL (Control group) No diagnosis of PDAC, not presenting with pancreatitis	(2) PANC (Pancreatitis group) No diagnosis of PDAC, presenting with pancreatitis	(3) PDAC (PDAC group) Diagnosis of PDAC
Sample Group			
# of Samples (Lot)	10 (--)	5 (A1)	15 (B,9)
Age	Mean: N/A Median: N/A Range: N/A	Mean: 64 Median: 67 Range: 24-83	Mean: 62.5 Median: 70 Range: 25-82

- Label-Free Quantification (LFQ) intensity data collected from LC-MS for each sample group was processed and pre-filted using MaxQuant and Perseus.

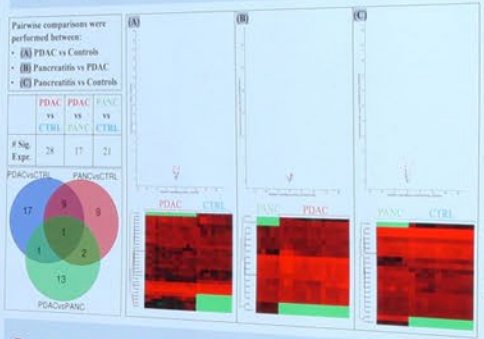
Identified proteins		
Complete expression	Uncertain expression	No expression
≥ 70% of sample replicates are Expressed	Between 30% and 70% of replicates are Expressed	≤ 30% of sample replicates are Expressed
Gap-filled according to Normal Distribution	Excluded	Replaced with values (0)

- Pairwise comparisons (PDAC vs. Controls, PDAC vs. Pancreatitis, Pancreatitis vs. Controls) were performed using Student's T-Test to determine which proteins were significantly expressed across each pair.
- Significantly expressed proteins for each pair were mapped and analyzed using pathway enrichment analysis through Ingenuity Pathway Analysis (IPA) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

Data Analysis – Student's T-Test, Hierarchical Clustering

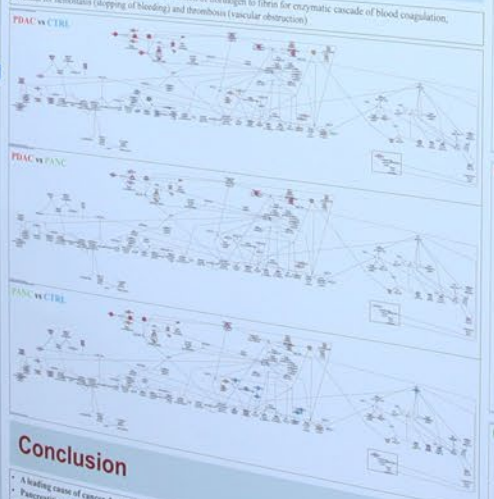
Pairwise comparisons were performed between:
- (A) PDAC vs Controls
- (B) Pancreatitis vs PDAC
- (C) Pancreatitis vs Controls

	PDAC vs CTRL	PDAC vs PANC	PANC vs CTRL
# Sig. Expr.	28	17	21



Results – Formation of Fibrin Clot (Clotting Cascade)

Formation of Fibrin (Clotting Cascade): conversion of fibrinogen to fibrin for enzymatic cascade of blood coagulation, essential for hemostasis (stopping of bleeding) and thrombosis (vascular obstruction)



Conclusion

- A leading cause of cancer death, PDAC h
- Pancreatitis not only sho
- In this stu

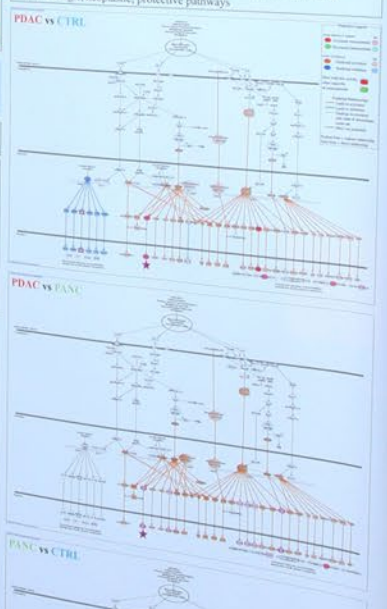
Results – Enriched Pathways

Top 5 Pathways

PDAC vs CTRL	PDAC vs PANC	PANC vs CTRL
1.LXR/RXR Activation	1.Acute Phase Response Signaling	1.Formation of Fibrin Clot (Clotting Cascade)
2.DHCR24 Signaling Pathway	2.Complement cascade	2.Response to elevated platelet cytosolic Ca^{2+}
3.Formation of Fibrin Clot (Clotting Cascade)	3.Integrin signaling	3.Extrinsic Prothrombin Activation Pathway
4.Response to elevated platelet cytosolic Ca^{2+}	4.Regulation of TLR by endogenous ligand	4.LXR/RXR Activation
5.Coagulation System	5.Integrin cell surface interactions	5.Coagulation System

Results – Acute Phase Response Signaling

Acute Phase Response Signaling: Rapid inflammatory response
Notable protein: Serum amyloid A (SAA)★
- Apolipoprotein; regulator of cell-cell communication, inflammatory, immunologic, neoplastic, protective pathways



Investigating the role of a novel long noncoding RNA in Epstein-Barr virus (EBV) infection and associated lymphomagenesis

Giancarlo Lara[1,#], Hirotomo Dochi[2,3,#], Janardhan Avilala[2,3,#], Hong Liu[2,3], and Zhen Lin[2,3]
[1]Louisiana State University, [2]Tulane University Health Sciences Center and Tulane Cancer Center, [3]Louisiana Cancer Research Center
#These authors contributed equally.

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Introduction

Satellite DNAs are tandem repetitive noncoding DNA sequences and constitute ~6% of the human genomes[1]. They serve as the main ... for heterochromatin and centromeres and have long ... al support for chromatin at the DNA level. ... iding blocks can be actively transcribed ... oduce non-polyadenylated satellite ... tion. Intriguingly, several studies ... oded by the largest satellite ... an cancer[2, 3]. We recently ... ouble-stranded breaks ... SatRNAs in both normal ... nd that DSB-induced α- ... nd thus is involved in ... nesis. Together, these ... RNAs in the process of ... tumor virus identified and ... annually[4-6]. EBV is ... malignant diseases ... EBVaL). To date, EBVaL patients ... survival and progression-free ... y patients succumb to this deadly ... hijack the ATM-mediated DNA ... self-preservation and ... ore the relationships ... its associated

Materials and Methods

Generation of α-SatRNAs expression vectors. The cDNA for α-SatRNAs was first generated by *in vitro* synthesis (Genscript). The cDNA was further amplified by PCR and specific restriction sites were added to both ends of the cDNA fragment. Following restriction digestion, the cDNA for α-SatRNAs was cloned into both retroviral and lentiviral vectors. The established α-SatRNAs expression vectors were purified from the transformed bacteria cells. Sanger sequencing analyses were then performed to verified the plasmid sequences.

***In vitro* Transfection.** Human embryonic kidney (HEK) 293 cells were transiently transfected with the plasmids using calcium phosphate transfection method.

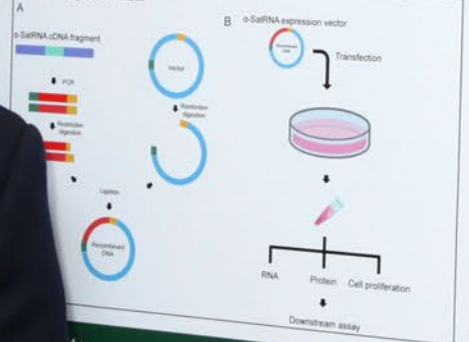
Quantitative reverse transcription PCR (RT-qPCR).

Cell proliferation assay. Cell proliferation was examined by the CellTiter assay (Promega).

Western blotting analysis.

RNAScope assay. Probes targeting α-SatRNA transcripts were designed and synthesized by ACDBio. To detect α-SatRNA transcript at the single-cell level, RNAScope assay was conducted.

Figure 2. Project overview.



ts

...ate the biological function of α-SatRNAs, we successfully ... number of α-SatRNA expression vectors (Figure 3).

...RNAs expression vectors were transiently transfected into 293 ...ssion of exogenous α-SatRNAs was confirmed by RT-qPCR ...4B).

...tion was analyzed by CellTiter assay (Figure 4C. Western blot ... an elevated level of γH2AX in cells expressing ComSat1x/3x

...ducted RNAscope ...

Figure 3. Establishment of α-SatRNA expression systems.

Figure 4. Transient expression of α-SatRNAs in 293 cells.

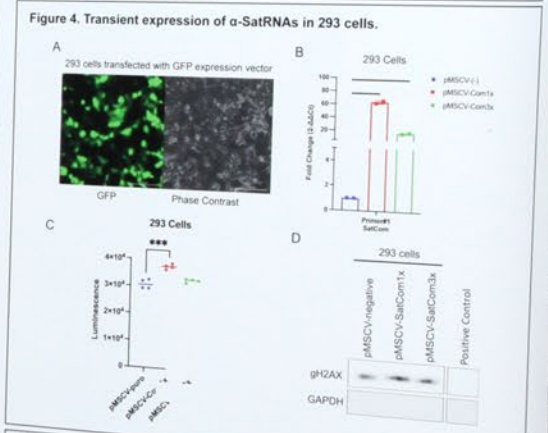
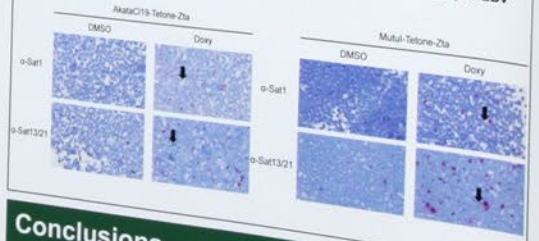


Figure 5. Increased levels of α-SatRNAs in lymphoma cells carrying lytic EBV infection.



Conclusions

Genetic Mutations in Familial ...: Insights & Implications

...e Bencaz MSPH, and Diptasri Mandal PhD
...C Department of Genetics

Methods

Conclusions & Future Directions

Interpretations of Results

- Prevalence of the EGFR and ALK gene mutations amongst study participants varied by about 5% each from what they were reported to be in literature
- Those with EGFR mutation
  - Higher number of relatives with LC
  - Earlier age of onset
  - Different types of mutations were observed: exon 19 deletion, exon 20 insertion, and p.T790M mutation
  - ...rticipants tested positive for the ALK ...ion despite being one of the most ...n genes tested for

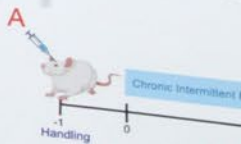
...reening panels for mutational ...ians ...mutational analysis

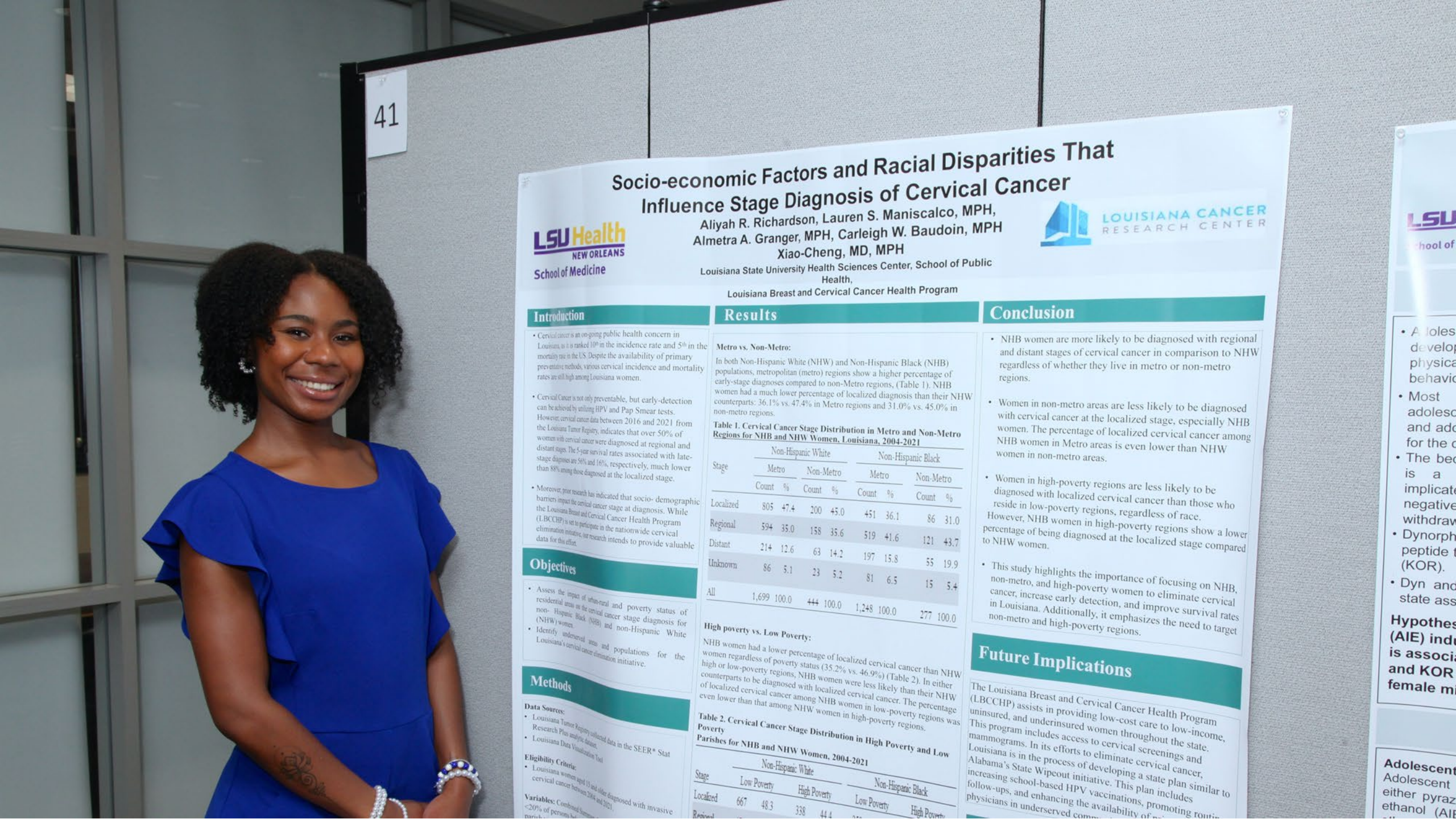
Introduct

- ...mans with alcohol use ... ...drawal (WD), which is ...
- T... neurobiology underlyi... t... ental area (VTA) and ...
- V... associated with rewa...
- C... associated with emot...
- F... us work has demons... d... alcohol WD in a rat ... p... ility that this circuit c...
- W... othesized that inhib... lik... havior of dependent ...
- To ... this hypothesis, we ... pro...ting VTA neurons and ...

Methods

- We first analyzed existing da... parameters influence behavi...
- We used a dual virus approa... neurons with an inhibitory de... designer drugs (DREADD). ... and a cre-dependent inhibito... injected into the VTA of adult ...
- To model alcohol dependenc... (CIE) to ethanol vapor paradi... hours a day and pure air for ...
- Blood alcohol concentrations ... 150-300 mg/dL
- Following 4 weeks of vapor e... behavior in an elevated plus ...
- To inhibit the VTA-CeA circuit, ... minutes prior to behavioral te...
- DCZ: designer drug that selec...
- Brains were sectioned to conf... inhibition of cells using phosp...

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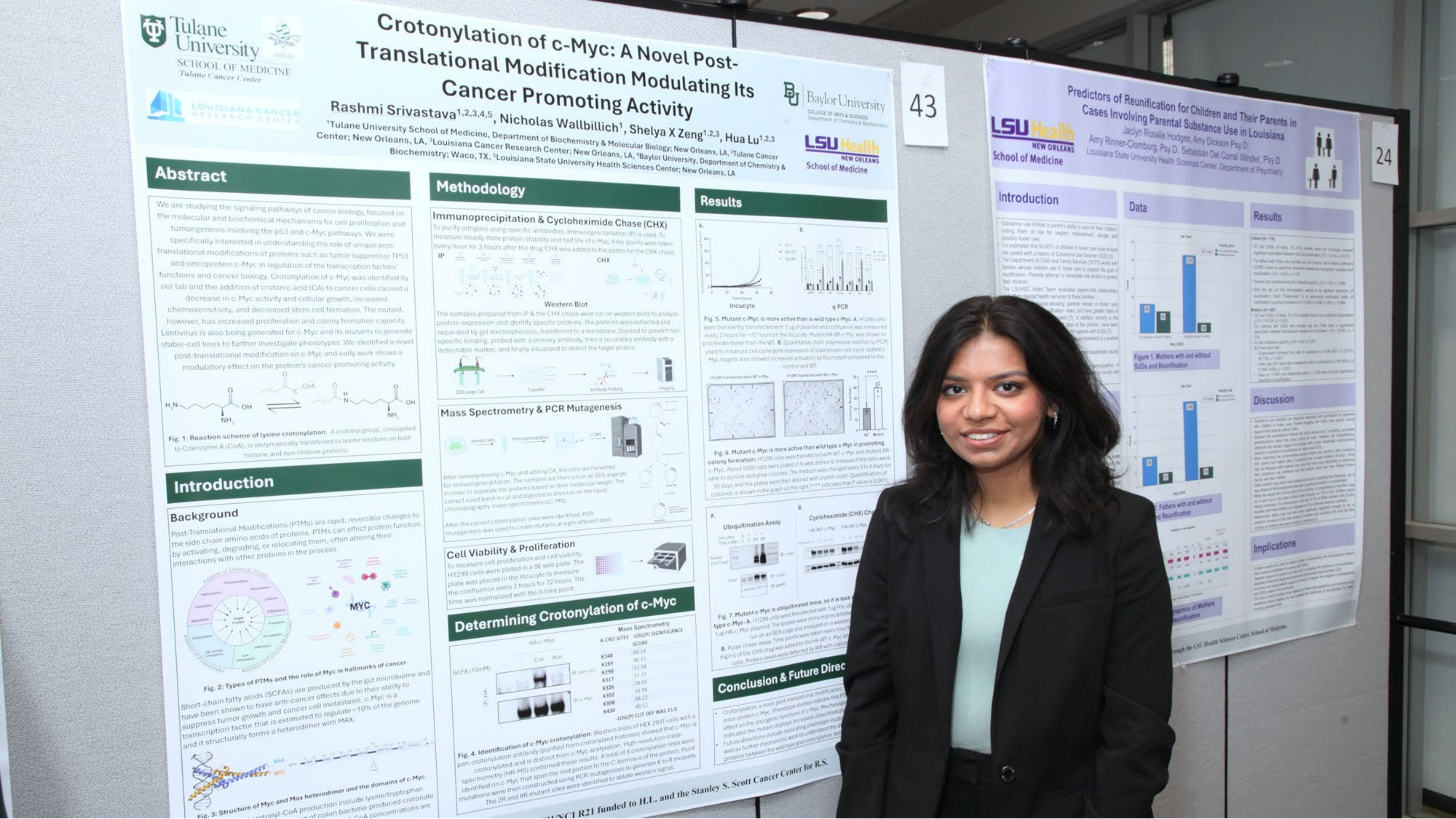
Socio-economic Factors and Racial Disparities That Influence Stage Diagnosis of Cervical Cancer
LSU Health NEW ORLEANS School of Medicine
Aliyah R. Richardson, Lauren S. Maniscalco, MPH,
Almetra A. Granger, MPH, Carleigh W. Baudoin, MPH
Xiao-Cheng, MD, MPH
Louisiana State University Health Sciences Center, School of Public Health,
Louisiana Breast and Cervical Cancer Health Program
LOUISIANA CANCER RESEARCH CENTER
Introduction
• Cervical cancer is an on-going public health concern in Louisiana, as it is ranked 10th in the incidence rate and 5th in the mortality rate in the US. Despite the availability of primary preventative methods, various cervical incidence and mortality rates are still high among Louisiana women.
• Cervical Cancer is not only preventable, but early-detection can be achieved by utilizing HPV and Pap Smear tests. However, cervical cancer data between 2016 and 2021 from the Louisiana Tumor Registry, indicates that over 50% of women with cervical cancer were diagnosed at regional and distant stages. The 5-year survival rates associated with late-stage diagnoses are 56% and 16%, respectively, much lower than 88% among those diagnosed at the localized stage.
• Moreover, prior research has indicated that socio- demographic barriers impact the cervical cancer stage at diagnosis. While the Louisiana Breast and Cervical Cancer Health Program (LBCCHP) is set to participate in the nationwide cervical elimination initiative, our research intends to provide valuable data for this effort.
Objectives
• Assess the impact of urban-rural and poverty status of residential areas on the cervical cancer stage diagnosis for non- Hispanic Black (NHB) and non-Hispanic White (NHW) women.
• Identify underserved areas and populations for the Louisiana's cervical cancer elimination initiative.
Methods
Data Sources:
• Louisiana Tumor Registry collected data in the SEER* Stat Research Plus analytic dataset.
• Louisiana Data Visualization Tool
Eligibility Criteria:
• Louisiana women aged 15 and older diagnosed with invasive cervical cancer between 2004 and 2021
Variables: Combined
<20% of persons
Results
Metro vs. Non-Metro:
In both Non-Hispanic White (NHW) and Non-Hispanic Black (NHB) populations, metropolitan (metro) regions show a higher percentage of early-stage diagnoses compared to non-Metro regions, (Table 1). NHB women had a much lower percentage of localized diagnosis than their NHW counterparts: 36.1% vs. 47.4% in Metro regions and 31.0% vs. 45.0% in non-metro regions.
Table 1. Cervical Cancer Stage Distribution in Metro and Non-Metro Regions for NHB and NHW Women, Louisiana, 2004-2021
Stage | Non-Hispanic White | | | | Non-Hispanic Black | | | |
Metro | | | Non-Metro | | Metro | | Non-Metro | |
Count | % | Count | % | Count | % | Count | %
Localized 805 47.4 200 45.0 451 36.1 86 31.0
Regional 594 35.0 158 35.6 519 41.6 121 43.7
Distant 214 12.6 63 14.2 197 15.8 55 19.9
Unknown 86 5.1 23 5.2 81 6.5 15 5.4
All 1,699 100.0 444 100.0 1,248 100.0 277 100.0
High poverty vs. Low Poverty:
NHB women had a lower percentage of localized cervical cancer than NHW women regardless of poverty status (35.2% vs. 46.9%) (Table 2). In either high or low-poverty regions, NHB women were less likely than their NHW counterparts to be diagnosed with localized cervical cancer. The percentage of localized cervical cancer among NHB women in low-poverty regions was even lower than that among NHW women in high-poverty regions.
Table 2. Cervical Cancer Stage Distribution in High Poverty and Low Poverty
Parishes for NHB and NHW Women, 2004-2021
Stage | Non-Hispanic White | | | | Non-Hispanic Black | |
Low Poverty | | High Poverty | | Low Poverty | High Poverty
Localized 667 48.3 338 44.4
Conclusion
• NHB women are more likely to be diagnosed with regional and distant stages of cervical cancer in comparison to NHW regardless of whether they live in metro or non-metro regions.
• Women in non-metro areas are less likely to be diagnosed with cervical cancer at the localized stage, especially NHB women. The percentage of localized cervical cancer among NHB women in Metro areas is even lower than NHW women in non-metro areas.
• Women in high-poverty regions are less likely to be diagnosed with localized cervical cancer than those who reside in low-poverty regions, regardless of race. However, NHB women in high-poverty regions show a lower percentage of being diagnosed at the localized stage compared to NHW women.
• This study highlights the importance of focusing on NHB, non-metro, and high-poverty women to eliminate cervical cancer, increase early detection, and improve survival rates in Louisiana. Additionally, it emphasizes the need to target non-metro and high-poverty regions.
Future Implications
The Louisiana Breast and Cervical Cancer Health Program (LBCCHP) assists in providing low-cost care to low-income, uninsured, and underinsured women throughout the state. This program includes access to cervical screenings and mammograms. In its efforts to eliminate cervical cancer, Louisiana is in the process of developing a state plan similar to Alabama's State Wipeout initiative. This plan includes increasing school-based HPV vaccinations, promoting routin follow-ups, and enhancing the availability of
physicians in underserved


Tulane University
SCHOOL OF MEDICINE
Tulane Cancer Center
LOUISIANA CANCER RESEARCH CENTER
Crotonylation of c-Myc: A Novel Post-Translational Modification Modulating Its Cancer Promoting Activity
Rashmi Srivastava1,2,3,4,5, Nicholas Wallbillich1, Shelya X Zeng1,2,3, Hua Lu1,2,3
Baylor University
LSU Health NEW ORLEANS School of Medicine
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Abstract
We are studying the signaling pathways of cancer biology, focused on the molecular and biochemical mechanisms for cell proliferation and tumorigenesis involving the p53 and c-Myc pathways. We were specifically interested in understanding the role of unique post-translational modifications of proteins such as tumor suppressor TP53 and oncoprotein c-Myc in regulation of the transcription factors' functions and cancer biology. Crotonylation of c-Myc was identified by our lab and the addition of crotonic acid (CA) to cancer cells caused a decrease in c-Myc activity and cellular growth, increased chemosensitivity, and decreased stem-cell formation. The mutant, however, has increased proliferation and colony formation capacity. Lentivirus is also being generated for c-Myc and its mutants to generate stable-cell lines to further investigate phenotypes. We identified a novel post-translational modification on c-Myc and early work shows a modulatory effect on the protein's cancer-promoting activity.
Fig. 1: Reaction scheme of lysine crotonylation.
Introduction
Background
Methodology
Immunoprecipitation & Cycloheximide Chase (CHX)
Western Blot
Mass Spectrometry & PCR Mutagenesis
Cell Viability & Proliferation
Determining Crotonylation of c-Myc
Results
Incucyte
q-PCR
Ubiquitination Assay
Conclusion & Future Direc
Predictors of Reunification for Children and Their Parents in Cases Involving Parental Substance Use in Louisiana
Jaclyn Rosalie Hodges, Amy Dickson Psy.D,
Amy Rinner-Clomburg, Psy.D, Sebastián Del Corral Winder, Psy.D
Louisiana State University Health Sciences Center, Department of Psychiatry
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LOUISIANA CANCER RESEARCH CENTER

Overcoming Multidrug Resistance in Breast Cancer: Targeting MRP Proteins for Enhanced Therapeutic Efficacy

Camryn Tims, Rajib Biswas, Treiveanna Jenkins, Anup Kundu PhD.
Biology Department, Xavier University; Tulane University Health Sciences Center

Introduction

In this project, the MDR proteins will be targeted as the major resistant protein in the cancer cell lines. When MRP-1/P-gp is highly expressed, it creates an extremely resistant cell environment for the breast cancer cells. This protein has the ability to dispel a huge list of anti-cancer drugs including doxorubicin. We anticipate that knocking down MRP-1/P-gp by siRNA encapsulated nanoparticles could enhance the delivery of doxorubicin (Dox) into the breast cancer cells. The preliminary study has been focused on the development of an aptamer-labeled nanoparticle system for effective delivery of MRP-1/P-gp siRNA into breast cancer cells.

Exp. Design

For targeted delivery, Aptamer-A6 has been used which can bind to Her-2 receptors on breast cancer cells. The particles were prepared by high pressure homogenization (HPH) using different amount of DOTAP, cholesterol, PLGA or PLGA-PEG and Mal-PEG. After siRNA encapsulation, the particles were incubated with aptamer-A6 for surface labeling. The liposomal particles were characterized for their size, surface charge and cytotoxicity. The delivery of siRNA into different breast cancer cells has been assessed by immunofluorescence and FACS analysis.

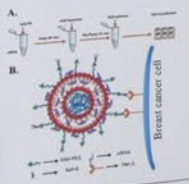


Figure 1. Schematic diagram showing the organization of the nanoparticles

Data

Figure 2. The expression of MRP-1 in 4T1-R and 4T1-S breast cancer cells by immunofluorescence.

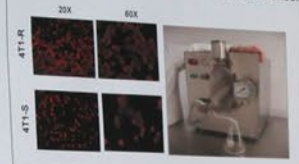


Figure 3. Comparison of particle size (a) and zeta potential (b) between blank and siRNA nanoparticles (with aptamer)

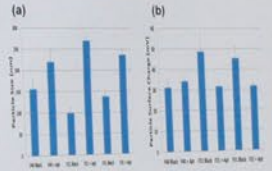
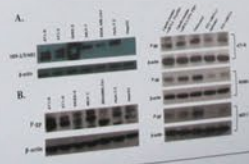


Figure 4. The expression of Her-2 and P-gp (left panel) and the knockdown of P-gp in 4T1-R, SKBR-3 and MCF-7 cells with/without aptamer labeled nanoparticles (right panel).



Assays

Western Blot

Western Blot tests were conducted to confirm the presence and size of the MRP-1 (Multi-drug Resistant Protein) in the dox-resistant cells.

qPCR

Additionally, qPCR tests were ran to identify and compare RNA concentrations amongst the 4T1-R and MDA-231 cell lines.

Conclusion

The prior study has shown that the uptake of Dox by Dox-resistant 4T1-R is significantly less than Dox-sensitive 4T1-S which is partly attributed to the higher expression of drug-efflux pump (i.e. ABC transporter proteins P-gp, MRP-1, BCRP etc.) on the surface of the resistant cells. The targeted knockdown of P-gp or MRP-1 has been enhanced when the particles carrying P-gp siRNA or MRP-1 siRNA, respectively were labeled with aptamer. Concurrently, the uptake of Dox into the Dox-resistant 4T1-R breast cancer cells has increased significantly when the MDR proteins were knockdown by appropriate siRNA-encapsulated aptamer-labeled nanoparticles.

References

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Determining the Efficacy of the Gardasil-9 Vaccine in HIV-Positive Individuals

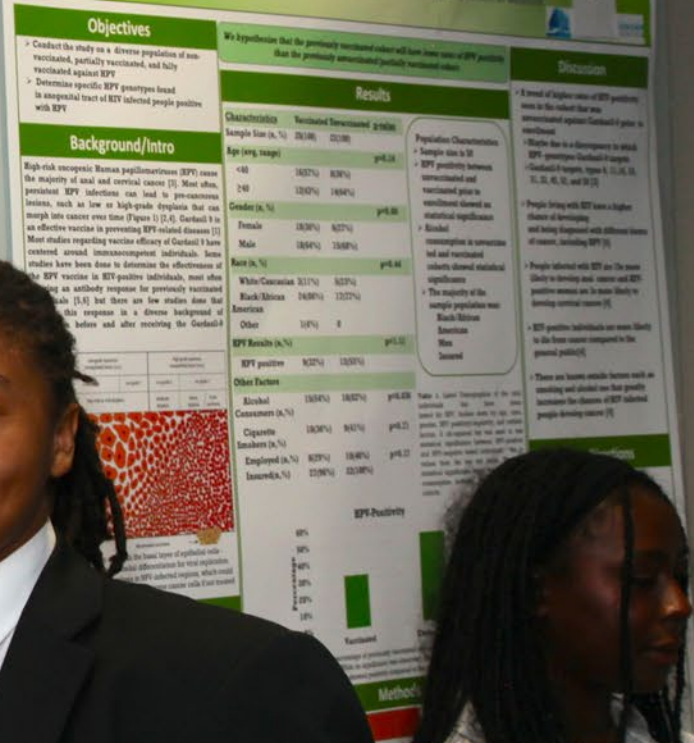
Tyra Washington, Ashley Winters, Jennifer Converse PhD, Michael Hagensee MD, PhD
LSUHSC ; Department of Microbiology, Immunology &Parasitology, Department of Medicine

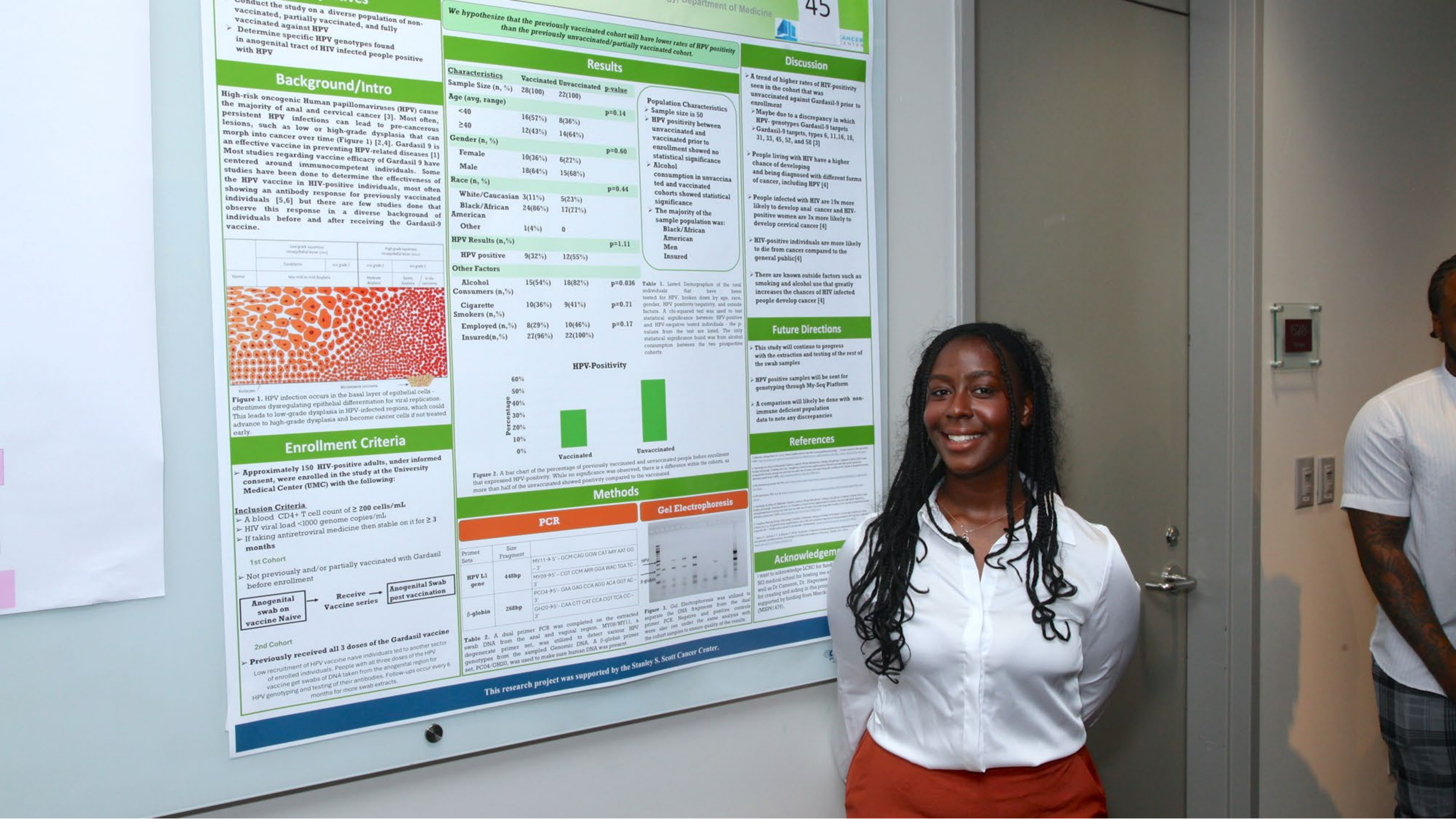
Objectives

Background/Intro

Results

Discussion




Conduct the study on a diverse population of non-vaccinated, partially vaccinated, and fully vaccinated against HPV
Determine specific HPV genotypes found in anogenital tract of HIV infected people positive with HPV
Background/Intro
High-risk oncogenic Human papillomaviruses (HPV) cause the majority of anal and cervical cancer [3]. Most often, persistent HPV infections can lead to pre-cancerous lesions, such as low or high-grade dysplasia that can morph into cancer over time (Figure 1) [2,4]. Gardasil 9 is an effective vaccine in preventing HPV-related diseases [1] Most studies regarding vaccine efficacy of Gardasil 9 have centered around immunocompetent individuals. Some studies have been done to determine the effectiveness of the HPV vaccine in HIV-positive individuals, most often showing an antibody response for previously vaccinated individuals [5,6] but there are few studies done that observe this response in a diverse background of individuals before and after receiving the Gardasil-9 vaccine.
Figure 1. HPV infection occurs in the basal layer of epithelial cells - oftentimes dysregulating epithelial differentiation for viral replication. This leads to low-grade dysplasia in HPV-infected regions, which could advance to high-grade dysplasia and become cancer cells if not treated early.
Enrollment Criteria
Approximately 150 HIV-positive adults, under informed consent, were enrolled in the study at the University Medical Center (UMC) with the following:
Inclusion Criteria
A blood CD4+ T cell count of ≥ 200 cells/mL
HIV viral load <1000 genome copies/mL
If taking antiretroviral medicine then stable on it for ≥ 3 months
1st Cohort
Not previously and/or partially vaccinated with Gardasil before enrollment
Anogenital swab on vaccine Naive
Receive Vaccine series
Anogenital Swab post vaccination
2nd Cohort
Previously received all 3 doses of the Gardasil vaccine
Low recruitment of HPV vaccine naive individuals led to another sector of enrolled individuals. People with all three doses of the HPV vaccine get swabs of DNA taken from the anogenital region for HPV genotyping and testing of their antibodies. Follow-ups occur every 6 months for more swab extracts.
We hypothesize that the previously vaccinated cohort will have lower rates of HPV positivity than the previously unvaccinated/partially vaccinated cohort.
Results
Characteristics Vaccinated Unvaccinated p-value
Sample Size (n, %) 28(100) 22(100)
Age (avg, range)
<40 16(57%) 8(36%) p=0.14
≥40 12(43%) 14(64%)
Gender (n, %)
Female 10(36%) 6(27%) p=0.60
Male 18(64%) 15(68%)
Race (n, %)
White/Caucasian 3(11%) 5(23%) p=0.44
Black/African American 24(86%) 17(77%)
Other 1(4%) 0
HPV Results (n,%)
HPV positive 9(32%) 12(55%) p=1.11
Other Factors
Alcohol Consumers (n,%) 15(54%) 18(82%) p=0.036
Cigarette Smokers (n,%) 10(36%) 9(41%) p=0.71
Employed (n,%) 8(29%) 10(46%) p=0.17
Insured(n,%) 27(96%) 22(100%)
Population Characteristics
Sample size is 50
HPV positivity between unvaccinated and vaccinated prior to enrollment showed no statistical significance
Alcohol consumption in unvaccinated and vaccinated cohorts showed statistical significance
The majority of the sample population was: Black/African American Men Insured
HPV-Positivity
Percentage
Vaccinated
Unvaccinated
Figure 2. A bar chart of the percentage of previously vaccinated and unvaccinated people before enrollment that expressed HPV-positivity. While no significance was observed, there is a difference within the cohorts, as more than half of the unvaccinated showed positivity compared to the vaccinated.
Methods
PCR
Gel Electrophoresis
Primer Sets
Size Fragment
HPV L1 gene 448bp
β-globin 268bp
Table 2. A dual primer PCR was completed on the extracted swab DNA from the anal and vaginal region. MY09/MY11, a degenerate primer set, was utilized to detect various HPV genotypes from the sampled Genomic DNA. A β-globin primer set, PC04/GH20, was used to make sure human DNA was present.
Discussion
A trend of higher rates of HIV-positivity seen in the cohort that was unvaccinated against Gardasil-9 prior to enrollment
Maybe due to a discrepancy in which HPV- genotypes Gardasil-9 targets
Gardasil-9 targets, types 6, 11,16, 18, 31, 33, 45, 52, and 58 [3]
People living with HIV have a higher chance of developing and being diagnosed with different forms of cancer, including HPV [4]
People infected with HIV are 19x more likely to develop anal cancer and HIV-positive women are 3x more likely to develop cervical cancer [4]
HIV-positive individuals are more likely to die from cancer compared to the general public[4]
There are known outside factors such as smoking and alcohol use that greatly increases the chances of HIV infected people develop cancer [4]
Future Directions
This study will continue to progress with the extraction and testing of the rest of the swab samples
HPV positive samples will be sent for genotyping through My-Seq Platform
A comparison will likely be done with non-immune deficient population data to note any discrepancies
References
Acknowledgements
This research project was supported by the Stanley S. Scott Cancer Center.
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