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TITLE: Minority Undergraduate Research in Prostate Cancer: Bridging Opportunities for Postbaccalaureate Education

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CONTRACTING ORGANIZATION: University of Delaware
Newark, DE 19716

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Four students, 3 from Delaware State University and 1 from Lincoln University, were recruited to participate in prostate cancer research in laboratories at the University of Delaware. In compliance with the aims of our grant the students each received intensive research training over the 10-week summer program. All students were required to participate in enrichment activities that spanned the scope of intellectual property, careers in medicine and science, as well as good research practice. Also in compliance with our aims, this grant sponsored three Health Disparity round table discussions that covered a range of issues in minority health. These discussions included topics of access, economic status, racial profiling, provider perceptions/misconceptions and race-based medicine. All discussions were based on primary literature as a lead in to the topic followed by group discussions. All students presented posters in a research symposium with over 500 participants from UD, Wesley, UMBC and other regional schools. Selected students participated in regional competitions.

15. SUBJECT TERMS
Undergraduate students, minority, abstracts, awards

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Introduction:
Due to the extremely low levels of minority faculty and graduate students in the sciences, the DoD Majority Institution (MI) /Historically Black College and University (HBCU) program was intended to foster and promote the interest of minority students in basic science and research by partnering one or more HBCU with a sponsoring MI. In Delaware, this has been accomplish by coordinating student recruitment from Delaware State University and Lincoln University to perform funded summer research in prostate cancer laboratories at the University of Delaware. Our Aims were to 1) offer a 10-week summer research program to five qualified minority students, 2) Offer a summer enrichment program to these students and 3) offer activities and extended research at the participating HBCUs during the following academic year.

Body:
In compliance with Aim 1, and upon the recommendation of the faculty campus coordinators at Delaware State University (Dr. Cindy van Golen) and Lincoln University (Susan Safford), 4 students from DSU and 1 student from Lincoln University were chosen for admission into the University of Delaware's training program in Prostate Cancer after being interviewed by prospective faculty mentors at UD. Our HBCU facilitators have been having an increasingly difficult time with this task due to high demand, or competition from other funded summer programs recruiting minority students nationally.

Student	**School**	**UD Mentor**	**Project**
Jhoneil Cooper	DSU	John Koh	Soft Agar Colony Formation Assays with LNCaP and CWR22 in the presence of PLM1, PAN52 and PLM6 compared to Bicalutamide
Jennifer Gray	DSU	Randy Duncan	
Odinaka Anyanwu	LU	Kirk Czymmek	The Role of the microRNA endonuclease Dicer in Sea Urchin Embryology
Navpreet Tung	DSU	Robert Sikes	HS-5 Bone Marrow Stromal Cell Conditioned Media may Promote Cell Cycle-Dependant Cell Death in Prostate Cancer through TGF-β Signaling
Ruth Joanis	DSU	Ken Van Golen	The Connection Between IGF-1 and the Activation of Different Rho GTPases and their Connection to Cell Invasion and Metastasis of Prostate Cancer

In compliance with Aim 2, students attended weekly seminars related to research http://www.udel.edu/chem/white/HHMI3/Summer10/S10enrichment.html. In addition our students attended discussion sessions on the topic of *Healthcare Disparities*. Prior to each session students were assigned to read both popular and scientific literature regarding the socio-economic or medical causes of healthcare bias. UD faculty from the Departments of Biological Sciences, Chemistry and Biochemistry moderated the discussions.

In compliance with Aim 3, Dr. van Golen and Dr. Usher presented seminars at DSU and Lincoln respectively. One student, Navpreet Tung, continued to perform bench science at UD through the next three semesters.

Key Research Accomplishments:
The students in this program were expected to make significant progress in research over a 10 week period. Students instructing them in laboratory procedures that included basic liquid handling, safety, and use of technology and equipment required. Despite this, the amount of publishable data that each student collected during this short time is amazing. Additionally, students were instructed to journal their research experience to enhance their level of comfort of communicating what skills and techniques they learned as well as understanding the research project. At the end of the summer program, each student presented the results of their research at the University's undergraduate research symposium, which required the students to produce a written abstract and poster for presentation. The symposium was modeled after the Experimental Biology meeting, where posters and talks occurred simultaneously and where there was a plenary lecture by a Howard Hughes Medical Institute investigator http://www.udel.edu/chem/white/HHMI3/Summer10/S10enrichment.html.

Navpreet Tung received a first place in his division at the UMBC (University of Maryland Baltimore County) undergraduate Research symposium.

Reportable Outcomes:
Five posters and 1 regional competition award

Conclusions:
Our students frequently state that their summer experience has made them evaluate research as a career option. In many cases this has resulted in graduate school applications instead of vocational programs in Nursing or other health related field. The students leave excited and we have had many students who apply for a second year. This fact alone suggests that we have a viable, rewarding program that is not redundant or repetitious from year to year. We are producing students of quality from HBCUs who can compete regionally and win prizes in poster competitions based on their results.

References:
None

Appendices:
Enrichment program schedule (Summer 2010)
Abstracts-Used for multiple meetings as described above (5)
Posters with each student presenting at year-end symposium (5)
UMBC poster competition news article

SUMMER 2010 UNDERGRADUATE RESEARCH ENRICHMENT PROGRAM

Tentative

Wednesdays 4 to 5 PM 205 Brown Lab

Special Sessions Thursdays June 10 4-5:30 PM
June 24 & July 28 NOON-1:30 PM

HHMI Scholars, Peter White Fellows, Beckman Scholars, and others including Science and Engineering Scholars

DATE	PROGRAM
On-line On your own	***What do you need to know about Safety in the Research Laboratory?*** ***Please note:*** ***You need to have completed safety instructions in your research laboratory and/or on-line before you start work in a laboratory. If you have questions, contact Occupational Health and Safety.***
Thurs June 10 4:00-5:30 Gore Hall	***Undergraduate Research Ethics Conference*** Dr. Thomas Powers, Department of Philosophy and co-director of the Science, Ethics, and Public Policy program administered by the Delaware Biotechnology Institute, and graduate students
June 16	***What are you doing here this summer? Introduction to Research and issues you may encounter.*** Dr. Harold White (Dept. Chemistry & Biochemistry, Director UD's HHMI Undergraduate Science Education Program). Dr. David Usher (Dept. of Biological Sciences, Assoc. Dir. UD's HHMI Undergraduate Science Education Program)

June 23	*Student Voices. Students who have done undergraduate research for more than a year describe their experiences.* **Megan Kissig, Nick Marze, Michael Napolitano, Wuroh Timbo, and Justin DiAngelo** BS Biology '02, PhD UPenn '10 (F 2010 begins Asst. Prof. Cell Biology at Hofstra University.)
Thurs June 24 (optional)	*Dealing with America's Health Disparities Problem - Part I Socioeconomic and Cultural Factors* **Drs. David Usher**, **Robert Sikes** (Dept. of Biological Sciences), **Jacqueline Aldridge** (NUCLEUS Program), and Cynthia van Golen (Delaware State University), and **Susan Safford** (Lincoln University) **Special optional session in 243 Wolf Hall from 12-1:30 PM. Food Provided.** Readings: **Disparities and Discrimination in Health Care-an Introduction** **Health Care Disparities Reading List Abstracts**.
June 30	*Don't stop now-Other University opportunities?* **Susan Serra**, Service Learning Coordinator and **Katharine Kerrane**, Senior Associate Director, Honors Program, and undergraduate panelists discussing National and International Scholarship Opportunities, Semester Abroad, Service Learning, and related opportunities. (Goldwater, Marshall, Mitchell, Rhodes, and Truman Scholarships, Fulbright Fellowships)
July 7	*How are things going? Mid-Session Perspectives.* **Dr. David Usher** (Dept. of Biological Sciences, Assoc. Dir. UD's HHMI Undergraduate Science Education Program), **Dr. Harold White** (Dept. Chemistry & Biochemistry, Director UD's HHMI Undergraduate Science Education Program).
July 14	*How do I get into Graduate School? (Must attend this session and/or the July 21 session)* **Dr. David Usher**, (Dept. of Biological Sciences), **Dr. Melinda Duncan**, (Dept. of Biological Sciences), **Dr. Brian Bahnson**, (Dept. of Chemistry and Biochemistry) **Dr. John Pelesko**, Dept. of Mathematical Sciences)
	Dealing with America's Health Disparities Problem- Part II

July 20 Tuesday lunch (Optional)	***Race-based Medicine*** **Drs. David Usher**and **Robert Sikes** (Dept. of Biological Sciences), **Jacqueline Aldridge** (NUCLEUS Program), **Dr. Cynthia van Golen** (Delaware State University), **Dr. Susan Safford** (Lincoln University) **Special optional session in 243 Wolf Hall from 12-1:30 PM** Reading: Should Racial Profiling have a Role in Cancer Prognosis?
July 21	***Managing a career in science. What is it like to be a scientist in academia or industry?*** ***Career biographies from academic and industrial scientists.*** **Erica Selva** and **Kenneth van Golen**, Department of Biological Sciences, **Charles Riordan**, Department of Chemistry and Biochemistry, and **Easley Wallace, Jr. ,** Principal Investigator, DuPont.
July 28	***How to communicate your Results - Conferences (Talks and Posters)*** **Megan Kissig,** BS Biology**, and Michael Napolitano**, BS Biochemistry **Judging Rubrics for the ASBMB Undergraduate Poster Competition 2007** A good site for **instructions on poster preparation**. Another good site.
August 4	***How do I get into Medical or other professional Schools?*** ***(Must attend this session and/or the July 14 session)*** **Dr. David Usher** (Dept. of Biological Sciences), **Aivi Nguyen,** BS Biology '09 (Jefferson Medical School), **Obi Mmagu**, BS Biology '09 PCOM, **Christine Arenson, MD** BS Chemistry '86, Director, Division of Geriatric Medicine, Jefferson Medical School.
	Undergraduate Research and Service Celebratory Symposium

Aug 11

Catherine Drennan

Professor of Chemistry
Massachusetts Institute of Technology
HHMI Professor
HHMI Investigator
Teaching General Chemistry with a Biological Emphasis

Plenary Lecture:

Snapshots of Proteins in Action

11:15 A.M.
Clayton Hall

9:00 - 11:00 AM
(Participants and their Poster Assignments to be posted in August)

Student Talks and Posters Presentations

Clayton Hall

(Last year's program)

Program organized by David Usher [e-mail: dusher at udel.edu], Department of Biological Sciences
Page last updated: 22 July 2010 by Hal White [e-mail: halwhite at udel.edu], Department of Chemistry and Biochemistry

Jhoneil Cooper and John Koh

Soft Agar Formation Assays with LnCap and CRW22 cells in the presence of PLM1, PAN52, and PLM6 compared to Bicalutamide and Flutamide

Introduction

Prostate Cancer is the second leading cause of cancer death in men. As prostate tissue is dependent on androgens for growth, anti androgens used alone or in conjunction with inhibitors of testosterone biosynthesis have been used in the treatment of Prostate Cancer however, often cancer cells escape such androgen blockade therapies. Antiandrogen failure can be caused by incomplete AR inactivation by antiandrogens caused by androgen receptor mutations, androgen receptor overexpression and or cytokine signaling crosstalk are associated with antiandrogen failure. Antiandrogen failure often leads to a clinical phenomenon known as anti-androgen withdrawal syndrome wherein anti-androgen resistant patients show symptomatic improvements after cessation of anti-androgen treatment. To compare compounds with each other, as PLM1 is bad in clonogenic assays where as PLM6 is good. Also compare the cells that were plated and to compare the other reactions to that of Biclutamide. PLM6 and PAN52 will react better with cells to form more resistant colonies in the soft agar colony assay. We will observe a difference in the incidence of resistant colonies between antiandrogen naïve CWR22 cells compared to flutamide resistant LNCaP cells if our second generation antiandrogens are acting through a non-antiandrogen specific resistance pathway. This experiment entails the assessment of colony formation of LnCap and CRW22 cells within the presence of the above mentioned drugs including a control. Cells will be plated in 6 well plates and a soft agar assay will be carried out which can be described as trapping the cells within a gel while testing them with specific compounds. The cells will then be allowed to grow for a two week period during which pictures of the cells will be taken to determine their reaction to the experiment and to also gather the results of the experiment.

Jennifer Gray, Mary Boggs, and Randall Duncan

Bone is highly innervated by sensory and sympathetic neurons which most believe are primarily implicated in controlling vascular activity. While the claim that these nerve fibers are purposefully located to regulate blood flow, studies have revealed the possibility of these neurons involvement in regulating bone structure (source-MB). This theory is supported by the discovery that the removal of sympathetic nerve fibers from bone leads to disregulation of the bone remodeling process. This phenomenon is indicative that neurons communicate with bone cells directly. However, the manner by which this communication system occurs is unknown. Thus, this study's aim is to investigate the effects neurotransmitters may have on osteocyte activity by the use of calcium imaging techniques. MLO_Y4 cells were treated independently with 100µL of GABA, Epinephrine, and glutamate agonist NMDA and AMPA.

The Role of the microRNA Endonuclease Dicer in Sea Urchin Embryology

Odinaka C. Anyanwu1, Deborah H. Powell2, Jia L. Song3 and Kirk J. Czymmek2,3

1 Lincoln University of Pennsylvania, Oxford, PA 2Delaware Biotechnology Institute, Bio-imaging Center, University of Delaware, Newark, DE 3Department of Biological Sciences, University of Delaware, Newark, DE

Dicer is an RNaseIII type endonuclease and is responsible for the regulation of microRNA. MicroRNAs are non-coding RNAs about 22 nucleotides in length. Specifically in adenocarcinoma, a form of prostate cancer [1], several are found to be up-regulated and directly proportional to the severity of the cancer. Conversely, it has also been shown that down regulation of Dicer expression in mice showed an enhanced tumorgenesis phenotype [2]. Considering this strong link of Dicer to cancer as well as microRNAs implicated in other cancers such as lymphomas, breast cancer, lung cancer and more, [3] a thorough evaluation of how it is regulated and how alterations in its expression affect cells is needed. For this research, sea urchins were used as a model system to evaluate the role of Dicer during embryonic development. Due to its optical transparency, the sea urchin embryo is very well-suited for microscopy experiments allowing delineation of cell types expressing Dicer in the entire embryo. Furthermore, its full genome recently has been sequenced and found to have significant homology to many important proteins in vertebrate biology. The aims of this project were to develop a method for localization of Dicer and 3D rendering of intact embryos with the subsequent identification of specific cell-types. Preliminary data suggested that Dicer may have a specific asymmetrical localization pattern during early gastrula development, while later stages lacked asymmetrical distribution.

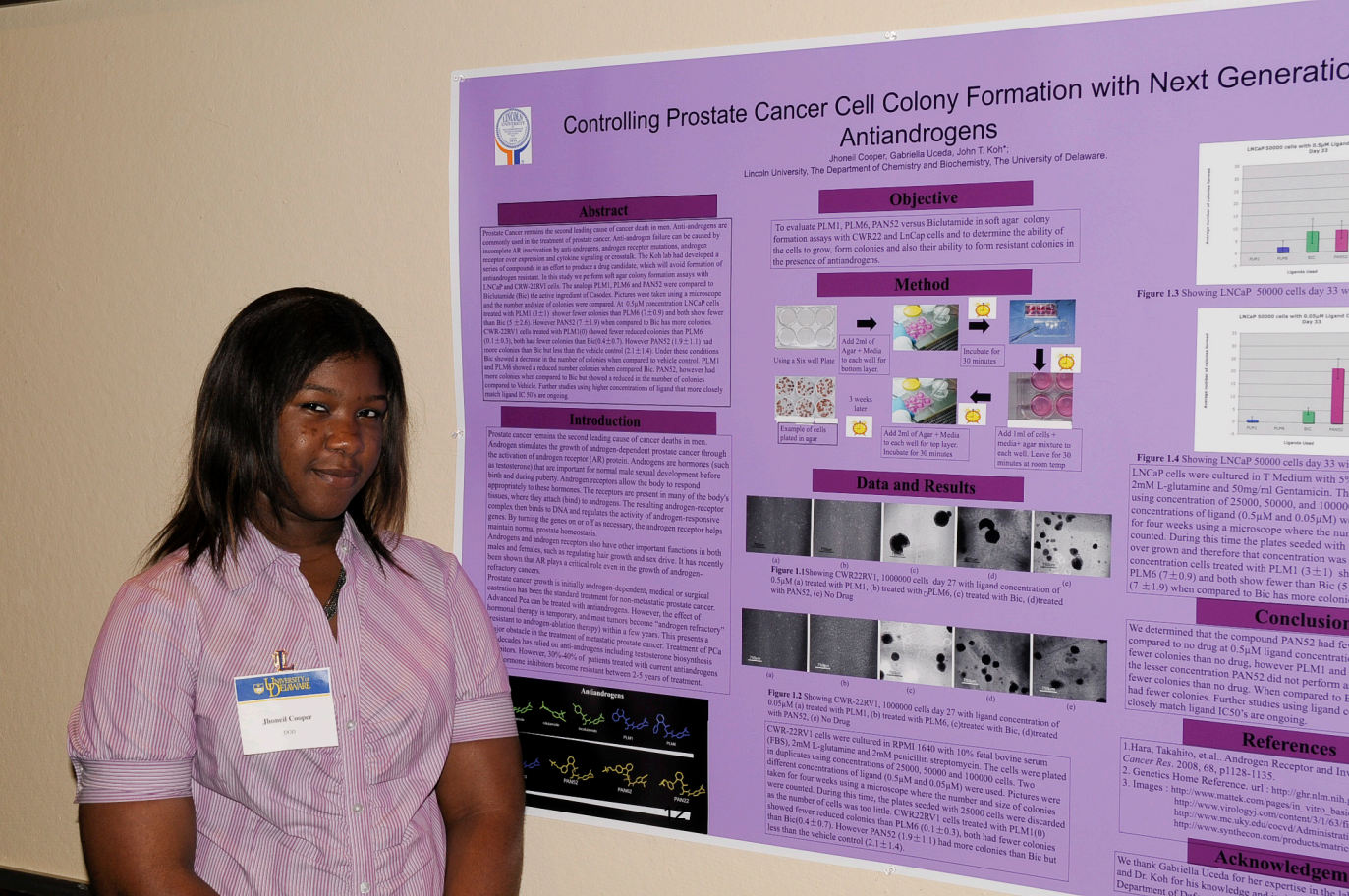
Navpreet Tung, Fayth L. Miles, Robert A. Sikes

Prostate cancer (PCa) is the second leading cause of cancer death in North American men. Aggressive PCa metastasizes to bone and is characterized by high levels of Transforming Growth Factor-β (TGF-β) in serum. We have shown that TGF-β reduces the growth of both bone-adapted and bone naïve PCa cell lines, although the mechanism has not been elucidated. Additionally, we have shown that the human bone marrow stromal cell line, HS-5, secretes a factor toxic to PCa cells, leading to increased cell death. This hostile bone stroma: PCa interaction is mediated through a TGF-β family member, as it is abrogated by SB-431542, an inhibitor of TGF-β type I receptors, ALK-4, -5, and-7. We hypothesized that this HS-5-induced cell death may be specific to the DNA synthesis phase of the cell cycle. Thus, in order to elucidate how and when TGF-β signaling stunts cell growth, we sought to 1) examine levels of potential TGF-β-regulated cell cycle proteins using western blotting and 2) measure potency of HS-5-induced death at specific phases of the cell cycle using flow cytometry and live/dead analysis. Our results indicate TGF-β downregulates cyclins and stimulates Smad phosphorylation, which correlates with decreased cell growth. Further, HS-5 conditioned media induces the highest levels of toxicity as PCa enters S phase. These findings demonstrate that bone colonization is a dynamic reciprocal interaction between bone stromal and PCa cells mediated, in part, through paracrine signaling by TGF-β family members resulting in either rejection or dormancy in the early colonization of the bone microenvironment.

Jonais RM, Dashner EJ, van Golen KL

Abstract

Signaling through the IGF-1 receptor was studied and it was found that multiple Rho GTPases were activated that were involved in cell survival, motility, and invasion. During a specific study the PC-3 cell line was used to observe the effect of anti IGF-1R treatment on the PC-3 cells. Rho C was inhibited through anti IGF-1R treatment and caused distinct morphological changes as well as changes in the expression level of proteins involved in the invasive capabilities of the metastatic PC-3 cell line. This initiated a study of IGF-1R signaling as well as the expression of Rho C in the LNCaP, C4-2, and the C4-2B (B4) cell lines, which are other metastatic prostate cancer lines that serve as an enhanced model of prostate cancer progression in humans. The results show that the expression of Rho C increases with the level of metastatic capability of prostate cells; the more metastatic cell lines, C4-2 and the C4-2B, have a higher expression of Rho C then the LNCap cell line. In the B4 cell line, a decrease in the activation Akt is shown when cells are treated with anti IGF-1R treatment. The B4 cell line also showed a decrease in growth when treated with an antibody targeted against the IGF-1R. It is concluded that anti IGF-1R treatment decreases the activation of Akt and that if either Akt or Rho C are inhibited, it may lead to a decrease in metastatic capability of prostate cancer cells. It is also concluded that when the B4 cell line is treated with 3B7, growth is decreased.


Controlling Prostate Cancer Cell Colony Formation with Next Generatio
Antiandrogens
Jhoneil Cooper, Gabriella Uceda, John T. Koh*;
Lincoln University, The Department of Chemistry and Biochemistry, The University of Delaware.
Abstract
Prostate Cancer remains the second leading cause of cancer death in men. Anti-androgens are commonly used in the treatment of prostate cancer. Anti-androgen failure can be caused by incomplete AR inactivation by anti-androgens, androgen receptor mutations, androgen receptor over expression and cytokine signaling or eroastak. The Koh lab had developed a series of compounds in an effort to produce a drug candidate, which will avoid formation of antiandrogen resistant. In this study we perform soft agar colony formation assays with LNCaP and CRW-22RV1 cells. The analogs PLM1, PLM6 and PAN52 were compared to Bicluatmide (Bic) the active ingredient of Casodex. Pictures were taken using a microscope and the number and size of colonies were compared. At 0.5μM concentration LNCaP cells treated with PLM1 (2±1) shower fewer colonies than PLM6 (7±0.9) and both show fewer than Bic (5 ±2.6). However PAN52 (7 ±1.9) when compared to Bic has more colonies. CWR-22RV1 cells treated with PLM1(0) showed fewer reduced colonies than PLM6 (0.1±0.3), both had fewer colonies than Bic(0.4±0.7). However PAN52 (1.9±1.1) had more colonies than Bic but less than the vehicle control (2.1±1.4). Under these conditions Bic showed a decrease in the number of colonies when compared to vehicle control. PLM1 and PLM6 showed a reduced number colonies when compared Bic. PAN52, however had more colonies when compared to Bic but showed a reduced in the number of colonies compared to Vehicle. Further studies using higher concentrations of ligand that more closely match ligand IC 50's are ongoing.
Introduction
Prostate cancer remains the second leading cause of cancer deaths in men. Androgen stimulates the growth of androgen-dependent prostate cancer through the activation of androgen receptor (AR) protein. Androgens are hormones (such as testosterone) that are important for normal male sexual development before birth and during puberty. Androgen receptors allow the body to respond appropriately to these hormones. The receptors are present in many of the body's tissues, where they attach (bind) to androgens. The resulting androgen-receptor complex then binds to DNA and regulates the activity of androgen-responsive genes. By turning the genes on or off as necessary, the androgen receptor helps maintain normal prostate homeostasis.
Androgens and androgen receptors also have other important functions in both males and females, such as regulating hair growth and sex drive. It has recently been shown that AR plays a critical role even in the growth of androgen-refractory cancers.
Prostate cancer growth is initially androgen-dependent, medical or surgical castration has been the standard treatment for non-metastatic prostate cancer. Advanced PCa can be treated with antiandrogens. However, the effect of hormonal therapy is temporary, and most tumors become "androgen refractory" resistant to androgen-ablation therapy) within a few years. This presents a major obstacle in the treatment of metastatic prostate cancer. Treatment of PCa decades has relied on anti-androgens including testosterone biosynthesis tors. However, 30%-40% of patients treated with current antiandrogens hormone inhibitors become resistant between 2-5 years of treatment.
Antiandrogens
PLM1
PLM6
PAN52
Objective
To evaluate PLM1, PLM6, PAN52 versus Biclutamide in soft agar colony formation assays with CWR22 and LnCap cells and to determine the ability of the cells to grow, form colonies and also their ability to form resistant colonies in the presence of antiandrogens.
Method
Using a Six well Plate
Add 2ml of Agar + Media to each well for bottom layer.
Incubate for 30 minutes
Add 1ml of cells + media+ agar mixture to each well. Leave for 30 minutes at room temp
Add 2ml of Agar + Media to each well for top layer. Incubate for 30 minutes
3 weeks later
Example of cells plated in agar
Data and Results
(a) (b) (c) (d) (e)
Figure 1.1Showing CWR22RV1, 1000000 cells day 27 with ligand concentration of 0.5μM (a) treated with PLM1, (b) treated with PLM6, (c) treated with Bic, (d)treated with PAN52, (e) No Drug
(a) (b) (c) (d) (e)
Figure 1.2 Showing CWR-22RV1, 1000000 cells day 27 with ligand concentration of 0.05μM (a) treated with PLM1, (b) treated with PLM6, (c)treated with Bic, (d)treated with PAN52, (e) No Drug
CWR-22RV1 cells were cultured in RPMI 1640 with 10% fetal bovine serum (FBS), 2mM L-glutamine and 2mM penicillin streptomycin. The cells were plated in duplicates using concentrations of25000, 50000 and 100000 cells. Two different concentrations of ligand (0.5μM and 0.05μM) were used. Pictures were taken for four weeks using a microscope where the number and size of colonies were counted. During this time, the plates seeded with 25000 cells were discarded as the number of cells was too little. CWR22RV1 cells treated with PLM1(0) showed fewer reduced colonies than PLM6 (0.1±0.3), both had fewer colonies than Bic(0.4±0.7). However PAN52 (1.9±1.1) had more colonies than Bic but less than the vehicle control (2.1±1.4).
LNCaP 50000 cells with 0.5μM Ligand Day 33
Average number of colonies formed
PLM1 PLM6 BIC PAN52
Ligands Used
Figure 1.3 Showing LNCaP 50000 cells day 33 w
LNCaP 50000 cells with 0.05μM Ligand C Day 33
Average number of colonies formed
PLM1 PLM6 BIC PAN52
Ligands Used
Figure 1.4 Showing LNCaP 50000 cells day 33 wi
LNCaP cells were cultured in T Medium with 5% 2mM L-glutamine and 50mg/ml Gentamicin. Th using concentration of 25000, 50000, and 10000 concentrations of ligand (0.5μM and 0.05μM) w for four weeks using a microscope where the nur counted. During this time the plates seeded with over grown and therefore that concentration was concentration cells treated with PLM1 (3±1) sh PLM6 (7±0.9) and both show fewer than Bic (5 (7 ±1.9) when compared to Bic has more coloni
Conclusion
We determined that the compound PAN52 had fe compared to no drug at 0.5μM ligand concentrati fewer colonies than no drug, however PLM1 and the lesser concentration PAN52 did not perform a fewer colonies than no drug. When compared to P had fewer colonies. Further studies using ligand c closely match ligand IC50's are ongoing.
References
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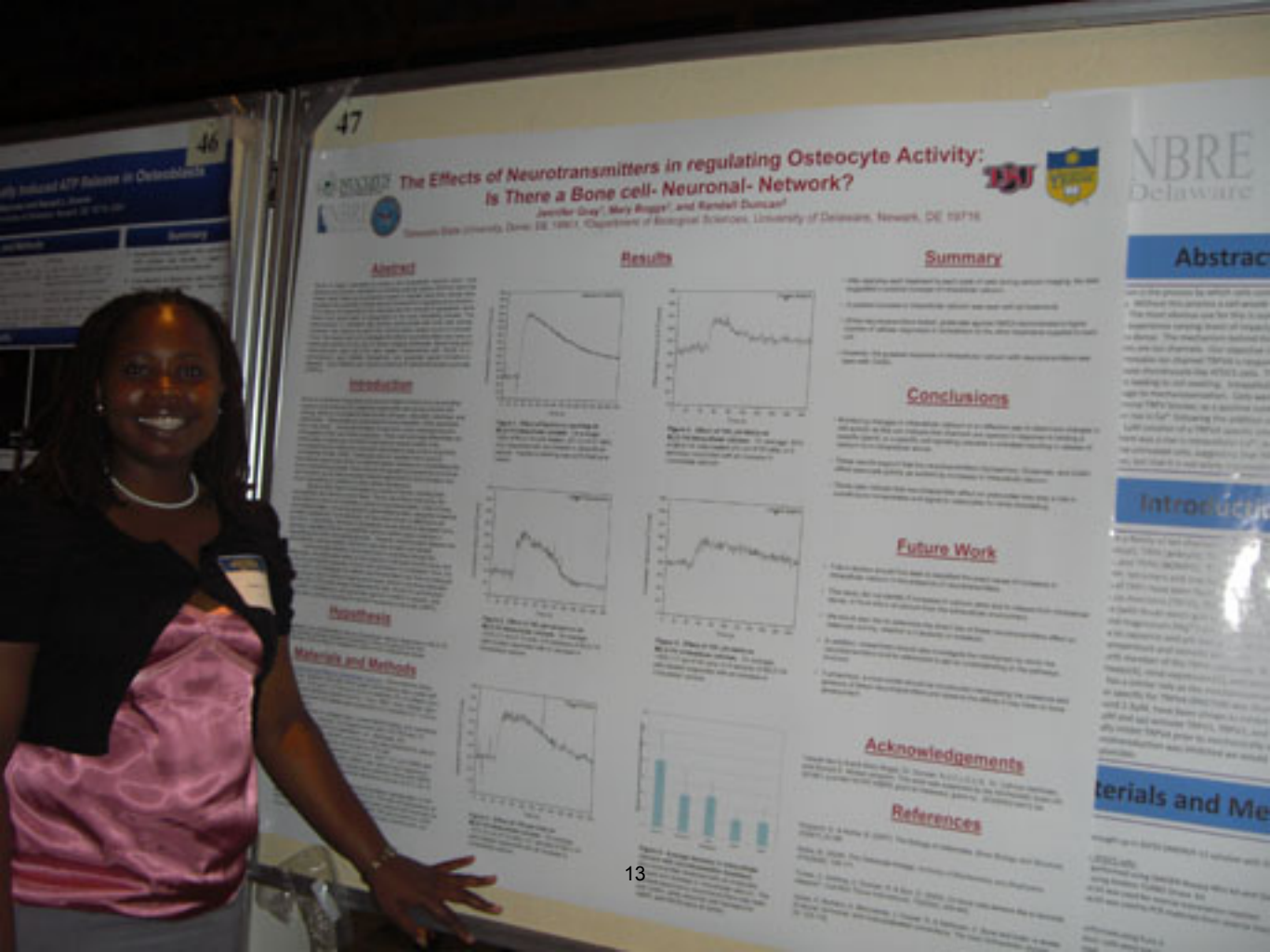
We thank Gabriella Uceda for her expertise in the l and Dr. Koh for his knowledge and
Department of Dr
Jhoneil Cooper
DGO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Is There a Bone cell- Neuronal- Network?
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Results
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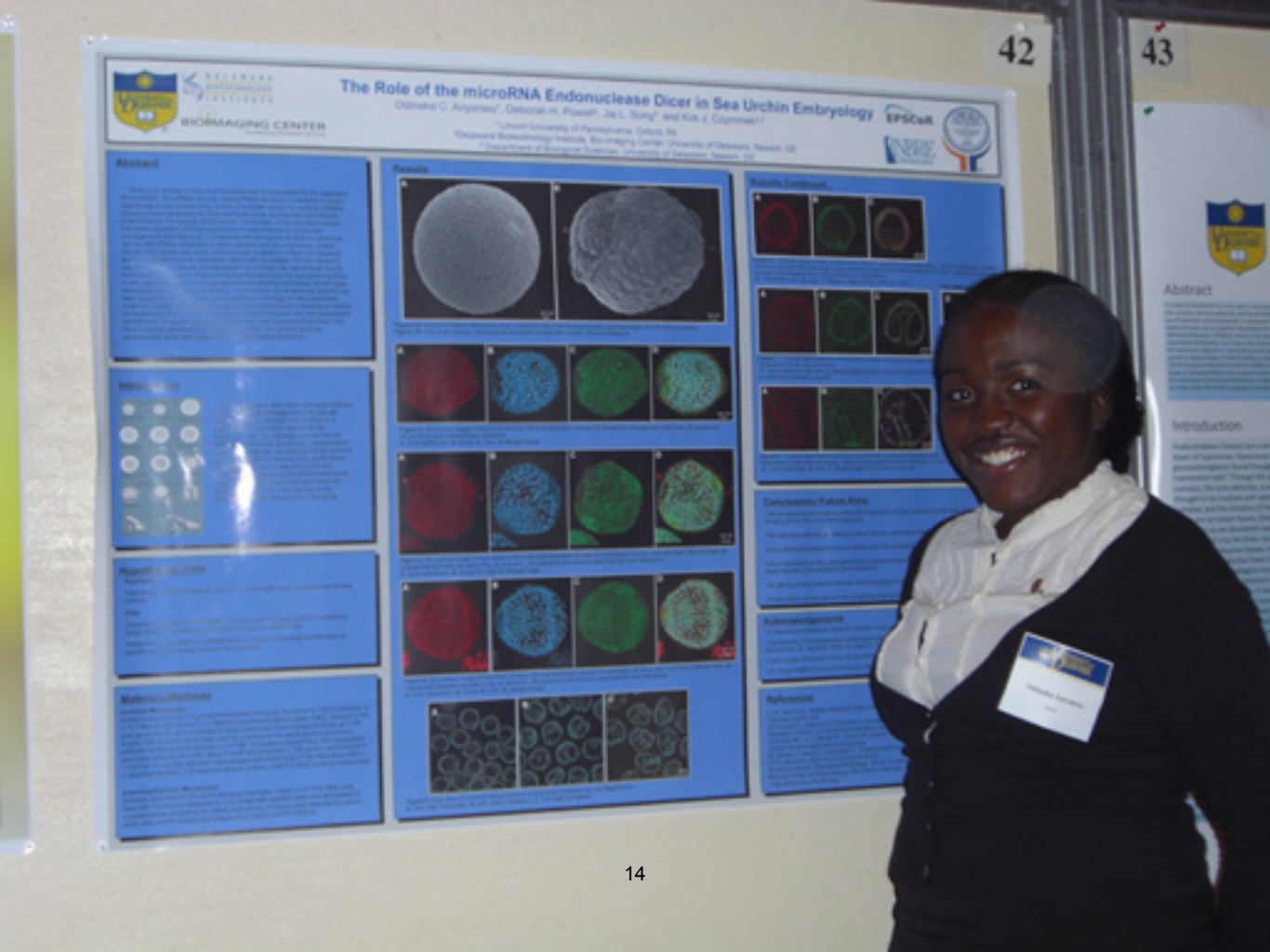
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The Role of the microRNA Endonuclease Dicer in Sea Urchin Embryology
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EPSCoR
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Abstract
Introduction

Complex Interactions Between Bone Stromal and Prostate Cancer Cells are Mediated Through TGF-β1 Signaling

Navpreet S. Tung, Fayth L. Miles, and Robert A. Sikes
Center for Translational Cancer Research, University of Delaware, Newark, DE 19716

stract

ng cause of cancer death in North American men. ed is characterized by high levels of Transforming ave shown that TGF-β reduces the growth of both ll lines, although the mechanism has not been hat the human bone marrow stromal cell line, HS-5, g to increased cell death. This hostile bone stroma- TGF-β family member, as it is abrogated by SB- ptors, ALK-4, -5, and-7. We hypothesized that this to the DNA synthesis phase of the cell cycle. Thus, GF-β signaling stunts cell growth, we sought to: 1) ted cell cycle proteins using western blotting and 2) th at specific phases of the cell cycle using flow results indicate TGF-β downregulates cyclins and correlates with decreased cell growth. Further, HS-5 levels of toxicity as PCa enters S phase. These ion is a dynamic reciprocal interaction between bone art, through paracrine signaling by TGF-β family or dormancy in the early colonization of the bone

oduction

cancer (PCa) can be eradicated with radical ical). However, the only treatment for advanced oval of circulating androgen through surgical or ults in regression of the cancer, it soon becomes aggressive phenotype, becoming more metastatic bone[1,2]. Once localized within the skeletal ty to alter homeostatic osteoconstitutive (bone formation extreme pain and poor prognosis[3]. Factors within the tokines, growth factors, mitogens, and tumor-bone te metastasis and bone colonization[3,4].

e are members of the transforming growth factor-beta regulate proliferation, differentiation, motility, adhesion and TGF- β3 belong to the TGF- β subfamily, which rmal tissue and early stage cancers, but transition to sses to the advanced and metastatic phase[5], an event on with surrounding stromal cells. The most established within this subfamily is TGF-β1. The TGF-β signaling F-β1 binds to TGF-β receptor type II (TBRII), a binding induces homodimerization of TBRII TBRII then ne residues of TGF-β receptor type I (TBRI) TBRI ero-tetrameric receptor complex. Phosphorylated TBRI ceptor bound proteins Smads 2/3. Once activated, 4 forming a transcription factor complex that rapidly to the promoter of over 500 diverse genes to activate or ng the androgen-sensitive LNCaP, and the androgen- e-derived derivatives C4-2 and C4-2B (respectively), we ath, and another TGF-β family member mediates death hanism of growth-regulation and relevance of cell cycle en elucidated.

ere to 1) Examine protein expression levels of the cell and p27kip in the presence of TGF-β1 through western of HS-5-induced death of PCa cells throughout various the toxicity is cell cycle dependent.

ls and Methods

% TCM defined serum for 24 hours. Cells were treated GF-β1 addition. Cells were

ycle
rs to synchronize and harvested in 6 hour increments and cell sorting to obtain a cell cycle profile.

ed Analysis
ed for 72 hours to synchronize. Cells were released ditioned medium in 6 hour increments ed with calcein AM and

Results

TGF-β1 Signaling Inhibits Growth in the LNCaP Progression Model

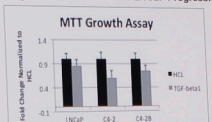

Figure 1. MTT cellular growth assay in the presence of TGF-β1. Examination of mitochondrial respiration levels indicates a decrease in cell number with TGF-β1 treatment in C4-2, C4-2B, and LNCaP, to a lesser extent. This suggests that TGF-β1 regulates growth in metastatic PCa cells.

TGF-β1 Downregulates Cyclin D and Upregulates p27kip Expression

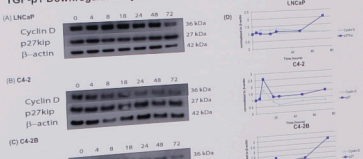

Figure 2. TGF-β1 slows growth in PCa cell lines **(A)** LNCaP, **(B)** C4-2, and **(C)** C4-2B after 72 hours of treatment. Cell cycle progressing protein, Cyclin D, is downregulated in C4-2 and C4-2B but not in LNCaP. Cyclin –dependent kinase inhibitor protein, p27, is upregulated in all 3 cell lines. **(D)** Graphical representation of western blot analysis.

pSmad2-Smad4 Complex Translocates to the Nucleus in the Presence of TGF-β1

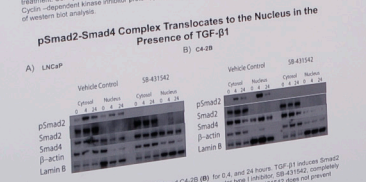

Figure 3. TGF-β1 treatments in LNCaP **(A)** and C4-2B **(B)** for 0, 4, and 24 hours. TGF-β1 induces Smad2 activation and nuclear translocation at 4 hours. TGF-β1 receptor type I inhibitor, SB-431542, completely abrogates Smad2 phosphorylation, thus restricting Smad2 to the cytosol. SB-431542 does not prevent translocation in LNCaP cells.

Cell Cycle Progression in a Synchronized Population

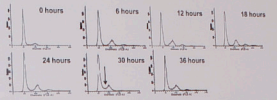

Figure 4. Flow cytometry analysis of a synchronized C4-2 population progressing uniformly through the cell cycle. Cells were serum starved for 72 hours to synchronize and released into 2.5% FBS. Cell cycle histograms indicate DNA synthesis initiated after 24 hours suggesting the cells took 1 day to recover from serum-starvation before entering S phase.

HS-5 Mediated Death is most Toxic During S Phase

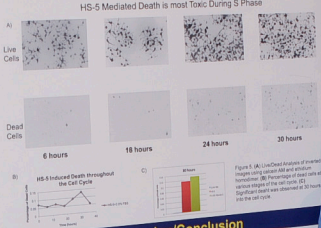

Figure 5. **(A)** Live/Dead Analysis of inverted images using calcein AM and ethidium homodimer. **(B)** Percentages of dead cells at various stages of the cell cycle. **(C)** Significant death was observed at 30 hours into the cell cycle.

Discussion/Conclusion

◇TGF-β1 inhibits growth through downregulation of Cyclin D in C4-2 and C4-2B cells and upregulation of p2 This potentially occurs through Smad2 and 4 activity.

◇More nuclear translocation of pSmad2 and Smad4 was evident in C4-2B than in LNCaP cells. This may correlate with increased and decreased levels of p27 and Cyclin D, respectively, observed in C4-2B.

◇HS-5 mediated cell death is most potent during the DNA synthesis phase of the cell cycle suggesting that t β signaling regulates this induced toxic effect.

◇An understanding of how bone stroma induces death in prostate cancer can lead to therapeutic approache target PCa. Future studies will aim to elucidate the HS-5 toxic factor and the signaling cascade through whic induces cell cycle-dependent death.

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Signaling Through the IGR-1R Mediates a Metastatic Phenotype in Prostate Cancer Cells

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INTRODUCTION

In the continuous quest for alternate therapeutic strategies to treat prostate cancer, scientists have already made groundbreaking discoveries about the progression of the disease. A very important discovery is that of the Insulin-Like Growth Factor (IGFs). It has also been proven that the IGFs largely regulate cell and tissue growth. Scientists have identified the role of type 1 Insulin-like Growth Factor (IGF-1) as having an impact on processes that control the metastatic capability of tumor cells. This includes cell proliferation, survival, migration, and invasion. A few years later scientists found that there is a close correlation between IGF-1 and the activation of multiple Rho GTPases and their involvement in the metastatic capabilities of cells, specifically prostate cancer. Rho GTPases are involved in cell survival, motility, and invasion. During this particular study, the PC-3 cell line was used, which is a benchmark cell line used to study prostate cancer. This study showed that there is a pathway that connects Rho GTPases and IGF-1, RhoC, a specific Rho GTPase that is expressed in the PC-3 cell line of prostate cancer and IGF-1 and how IGF-1 stimulated motility and invasion. The results showed that in the PC-3 cell line, when RhoC GTPase was inhibited there were specific morphological changes in the cells that caused a decrease in cell motility and invasion and the progression of invasion in the metastatic PC-3 cell line. The aim of my research project is to understand how RhoC GTPase becomes activated through the IGF-1 receptor. I will be studying the LNCaP, C4-2, and the C4-2B cell lines, which better imitate the progression of prostate cancer in a wider range of patients. The significance of this research is that if we can understand the process of the GTPase activation through the IGF-1R, it will lead to the next step, which is learning how to block activation of RhoC GTPases. If in fact these Rho GTPases are being activated through the IGF-1R, antibodies can be used against the IGF-1R to block the activation of these Rho GTPases. The goal of the treatment with the antibodies will severely compromise the metastatic capability of prostate cancer cells and make them easier to treat.

OBJECTIVES

- Understand signaling through the type I Insulin-Like Growth Factor Receptor (IGF-1R)
- Determine the level of expression of RhoC GTPase in the LNCaP-C4-2, and C4-2B lines
- Attempt to use antibodies directed against the IGF-IR to prevent the metastatic phenotype of prostate cancer cells.

METHODS

- Western Blotting: Samples were loaded into a gel and run for 1.5 hours. The gel was then transferred to a nitrocellulose membrane paper for a period of 1 hour. The blot was then taken and incubated with a blocking agent (4% BSA or 3% Milk in TBST) for 1 hour. The primary antibody was then added to the blot and incubated in the cold room overnight. The blot is then removed from the cold room and washed three times with secondary antibody for 5 minutes. The blot was repeated three times for 5 minutes. 2 hours for Chemi was added and incubated 1) Three minutes with 0.1% PBST. The blot was washed in the cold room again done in PBS for 5 minutes. After the blot was washed in the cold room again done in PBS for 5 minutes. After the blot was washed in the cold room and incubated for 1 min. of chemi developer for one minute. The blot was then imprinted on x-ray film.
- Cell Growth using a counter and Bio-Rad Plate reader: Cells were counted in counters using a counter and Bio-Rad Plate reader and incubated for a 24 hour period to allow adhesion to the cell plate. Next the cells were serum starved for 24 hours. MTT was then added to the plate. After the serum starvation for 24 hours, MTT was then added to the plate. After the plate was incubated for one hour, 100 µl of DMSO was added to dissolve the crystals completely dissolved and the plate was read by absorbance at 570 nm. The remaining plates were then treated every other day and then read at there respective days following the same procedure as the day one plate.

Figure 1: Signaling through the IGF-IR in PCa

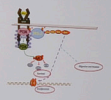


Figure 2: Signaling through the IGF-IR axis leads to increased survival, proliferation, migration and invasion of PCa cells.

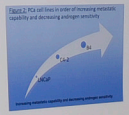


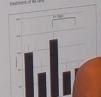
Figure 2: PCa cell lines in order of increasing metastatic capability and decreasing androgen sensitivity

Figure 3: Increased expression of RhoC GTPase in metastatic PCa cells

Figure 4: The activation of RhoC has a higher expression in the more metastatic PCa cells regardless of media type.

Figure 5: In the 66 prostate cancer cell line, wnt GF-1A treatment decreases the activation of akt, a protein involved in promoting cell survival.

Figure 6: Growth data for wnt IGF-1R treatment of 66 cells



RESULTS

SUMMARY AND CONCLUSIONS

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I would like to thank the Department of Biological Sciences and the Center for Translational Cancer Research for the various materials and resources that were used in the completion of this project.

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UD students excel at UMBC undergraduate research symposium

11:17 a.m., Nov. 8, 2010----Seven University of Delaware students, and one Delaware State University student who is doing research at UD, won top awards at the 13th annual Undergraduate Research Symposium in the Chemical and Biological Sciences at the University of Maryland, Baltimore County, on Oct. 30.

Supported by the National Institutes of Health (NIH), the research conference was devoted entirely to contributions from undergraduates from universities and colleges across the Mid-Atlantic region. Students presented the results of their work in chemistry, biology and at the chemistry-biology interface in poster sessions. All entries were judged in groups of about 5-7 posters with the two top-rated entries in each disciplinary group receiving awards.

Accompanying the UD students was Hal White, professor of chemistry and biochemistry and director of the UD Howard Hughes Medical Institute (HHMI) Undergraduate Science Education Program, which sponsored the students' travel to the event.

“This is the fifth year we have brought students to the UMBC Symposium, and every year the students have done extremely well,” White said. “Their success is a real feather in the cap for the Undergraduate Research Program at Delaware. Several of these students will be going on to present their work at the national Experimental Biology Meetings next April in Washington, D.C.”

Fourteen UD students and one DSU student participated:

- Erica Boetefuer, “The role of *ATG18* in signal transduction pathways during *Drosophila* development,” 2nd place, Biology Group 1L. Adviser Erica Selva.
- Michael Brister, “The structural characteristics of synthetically glycosylated Tau protein sequences,” 2nd place, Biochemistry Group 2P. Adviser Neal Zondlo.
- Amy Chevalier, “Trafficking patterns of adenosine A2A receptor,” Chemical Engineering. Adviser Anne Robinson.
- Kristofer Dewberry, “Determining the capacity of pulmonary cells to exit the lung during acute influenza virus infection,” Animal Science, for work at the University of Pennsylvania School of Medicine. Adviser Gudrun Debes.
- Timothy Gilpatrick, “Examining the binding properties of the enzyme LP-PLA2 and investigating its correlation with coronary heart disease,” Biochemistry. Adviser Brian Bahnson.
- Soma Jobbagy, “Characterization of next generation anti-androgens as potential prostate cancer therapeutics,” Biochemistry. Advisers Robert Sikes and John Koh.
- Matthew King, “Fibronectin appears in distinctive patterns in the lens of the eye,” 1st place, Biology Group 1K. Adviser Melinda Duncan.
- Sanjana Luther, “Comparing the immune response of C57BL6 and BALB/c mice infected with *Vibrio cholera,*” Biology. Adviser Michelle Parent.
- Chet Markwalter, “Elucidating mechanisms of heterologous neurokinin 2 receptor expression and trafficking in *S. cerevisiae,*” Chemical Engineering. Adviser Anne Robinson.
- Suranjit Mukherjee, “Synthesis of silver nanoparticles for use in an animal model,” 2nd place, Biology Group 1J. Adviser Anja Nohe.
- Tejal Naik, “Development of a peptide nucleic acid based siRNA delivery system,” 2nd place, Biochemistry Group 2N. Adviser Millicent Sullivan.
- Victoria Roop, “Characterization of the EMT response in CRYBB2PHILmutants,” 1st place, Biology Group 1I. Adviser Melinda Duncan.
- Robert Sheehan, “The effects of histone modification on lens fiber cell denucleation,” Biology. Adviser Melinda Duncan.
- Navpreet Tung (Delaware State University), “Complex interactions between bone stroma and prostate cancer cells is mediated through TGF-Beta signaling,” 1st place, Biology Group 2G. Adviser Robert Sikes.
- Devan Turner, “Vesicle formation through encapsulation of biologically-compatible ionic liquids,” 2nd place, Chemistry Group 2B, for work completed as an HHMI Exceptional Research Opportunities Program (EXROP) student with Isiah Warner at Louisiana State University.

University of Delaware students excelled at the Undergraduate Research Symposium at the University of Maryland, Baltimore County, Oct. 30. Pictured are, front row, from left, Michael Brister, Sanjana Luther, Amy Chevalier, Erica Boetefuer, Suranjit Mukherjee, Tejal Naik, Robert Sheehan and Devan Turner, and, back row, from left, Navpreet Tung (Delaware State University), Soma Jobbagy, Chet Markwalter, Kristofer Dewberry, Timothy Gilpatrick and Matthew King.

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The HHMI Undergraduate Science Education Program at UD has several components, one of which is to strengthen undergraduate research in the biomedical sciences. Last summer, the program supported 25 UD students working in faculty laboratories in biology, chemistry, biochemistry, chemical engineering, mathematics and physical therapy.

Earlier this year, UD was one of 50 research universities nationwide to receive a grant from the Howard Hughes Medical Institute (HHMI) for innovative programs to strengthen undergraduate and precollege science education. The four-year grant, which began Sept. 1, is the fifth HHMI award that UD has won.