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Medical College of Wisconsin
2011 Breast and Prostate Cancer Research Program Report
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MEDICAL COLLEGE OF WISCONSIN
DECEMBER 29, 2011

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BREAST AND PROSTATE CANCER RESEARCH STATE INCOME TAX CHECK-OFF PROGRAM REPORTING PERIOD JULY 1, 2010 - JUNE 30, 2011

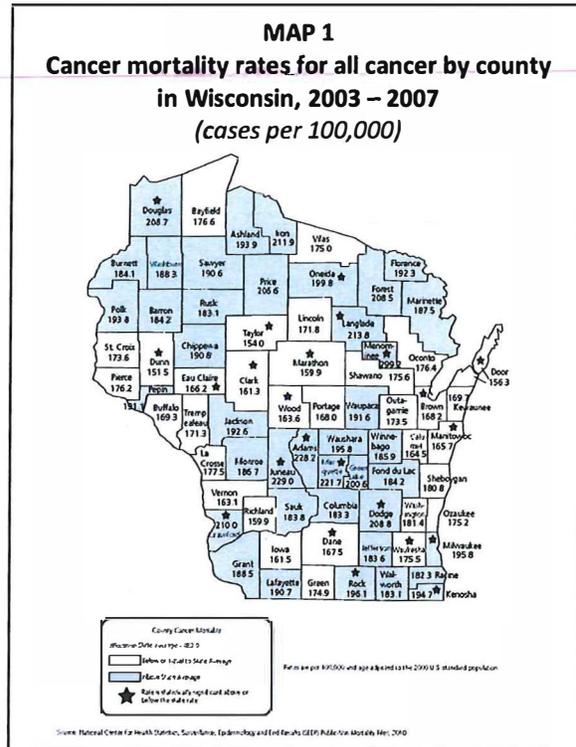
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A. BACKGROUND: UNITED STATES AND WISCONSIN STATISTICS

Malignant neoplasms (cancer) is the second leading cause of death in the United States and accounts for nearly 1 of every 4 deaths, slightly behind heart disease.¹ In 2011, about 1.5 million new cancer cases are expected to be diagnosed, and more than 1,500 Americans are expected to die per day from the disease.² The American Cancer Society estimates that over 30% of Americans now living will eventually develop cancer in their lifetime.

In Wisconsin this year, one resident will die of cancer each hour, and 81 Wisconsin residents will be told they have the disease (Map 1).^{3/4} Cancer is the leading cause of death for Wisconsin women ages 25-84 and for men ages 45-84. Cancer accounts for 24 percent of deaths among whites, 23 percent of deaths among African Americans, and 20 percent of deaths among both American Indians and Asians.⁵

Cancer causes, on average, one out of every four deaths in Wisconsin.



¹Miniño AM, Xu JQ, Kochanek KD. Deaths: Preliminary Data for 2008. National Vital Statistics Reports; vol 59 no 2. Hyattsville, MD: National Center for Health Statistics. 2010. http://www.cdc.gov/nchs/data/nvsr/nvsr59/nvsr59_02.pdf

²American Cancer Society – Cancer Facts and Figures 2011 <http://www.cancer.org/acs/groups/content/@epidemiologyandprevention/documents/document/acspc-029771.pdf>

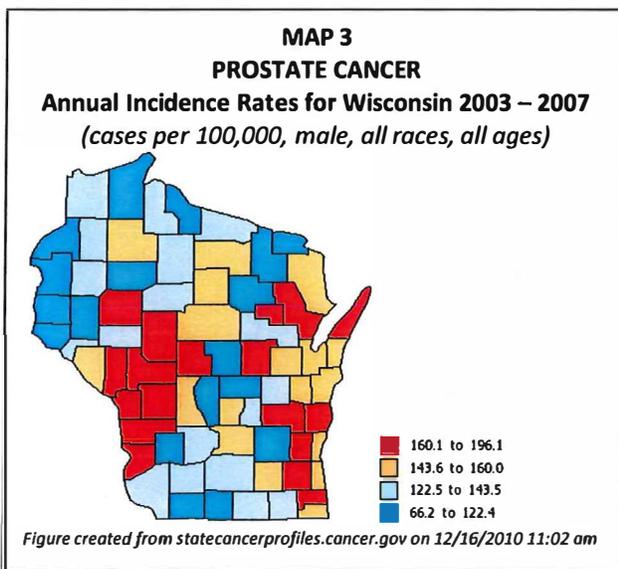
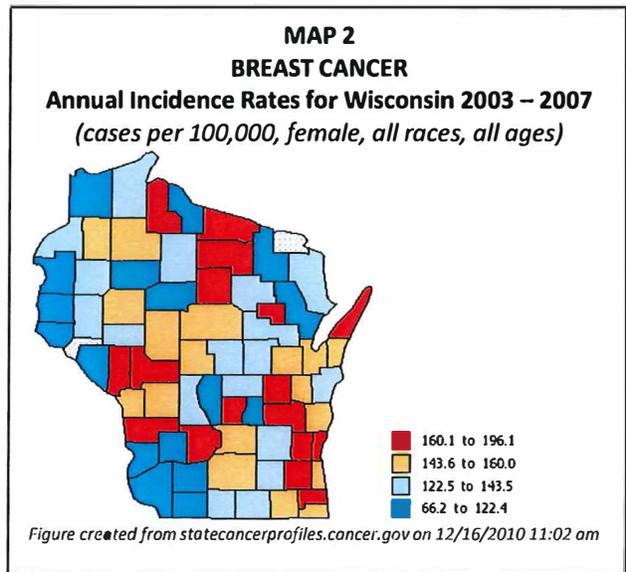
³Wisconsin Department of Health Services, Division of Public Health, Office of Health Informatics. *Wisconsin Deaths, 2008* (P-45368-08). October 2009.

<http://www.dhs.wisconsin.gov/deaths/pdf/08deaths.pdf> and http://www.wiscancer.org/documents/WIFactsFigures2011_Final.pdf

⁴US Cancer Statistics Working Group. United States Cancer Statistics: 1999-2007 Incidence and Mortality Web-based Report. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute 2010. www.cdc.gov/uscs

⁵Wisconsin Deaths – February 2011 – Office of Health Informatics, Division of Public Health, Wisconsin Department of Health Services

Breast cancer is the most common non-skin cancer in the United States and is the second leading cause of cancer death in women, after lung cancer. One in 8 American women will be diagnosed with breast cancer sometime during her lifetime.⁶ In Wisconsin, breast cancer remains the most frequently diagnosed cancer in women, representing 28% of all female cancer diagnoses (Map 2).⁷ The National Cancer Institute estimates that nearly 3 million women in the United States are currently diagnosed with or have survived breast cancer, over 230,000 women will be diagnosed with the disease in 2011, and over 41,000 of them will die from the disease. Of the nearly 4,000 Wisconsin women who are diagnosed with breast cancer each year, over 800 of them die from the disease. Importantly, breast cancer is not an exclusively female-related disease. More than 1,700 men are diagnosed with breast cancer each year in the United States and approximately 450 of them die from the disease.



Prostate cancer is the second most common type of cancer among men in the United States and is the most frequently diagnosed cancer among Wisconsin males, representing 28% of all cancers diagnosed between 2003 and 2007 (Map 3). Over 2 million American men who are currently diagnosed with or have survived prostate cancer are still alive today, approximately 240,000 men will be diagnosed with the disease in 2011, and it is estimated that over 33,000 of them will die from the disease. One in six men will be diagnosed with cancer of the prostate during his lifetime, and one man in 36 will die of this disease. Approximately 200,000 new cases of prostate cancer are diagnosed each year, 4,000 of which are in Wisconsin. Over 600 of those Wisconsin men will die from their disease.

⁶ Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, Ruhl J, Howlander N, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Cronin K, Chen HS, Feuer EJ, Stinchcomb DG, Edwards BK (eds). *SEER Cancer Statistics Review, 1975-2007*, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2007/, based on November 2009 SEER data submission, posted to the SEER web site, 2010. <http://www.cancer.gov/>

⁷ American Cancer Society – Wisconsin 2011 Facts and Figures http://www.wicancer.org/documents/WIFactsFigures2011_Final.pdf

B. WISCONSIN STATE CANCER RESEARCH TAX CHECK-OFF PROGRAM

The State of Wisconsin established a Breast Cancer Research State Income Tax Check-Off Program under Assembly Bill 351 in 2003 to fund breast cancer research and a Prostate Cancer Research Program funded through the same mechanism in 2005 with the passage of Assembly Bill 1087. Under the acts, every individual filing a Wisconsin income tax return who has a tax liability or who is entitled to a tax refund, may provide any amount of additional payment or refund go to the breast and/or prostate cancer research programs.

Under the provisions of 2003 Wisconsin ACT 176 and 2005 Wisconsin ACT 460, the amount received under the State Income Tax Check-Off, after administrative expenses, are divided evenly between the Medical College of Wisconsin (MCW) and the University of Wisconsin Comprehensive Cancer Center. The law requires both entities to use the money for breast and prostate cancer research and to report annually on the breast and prostate cancer research projects conducted during the previous year. These funds may not be used to supplant funds available from other sources.

The Medical College of Wisconsin received its sixth appropriation from the Breast Cancer Research State Income Tax Check-off Program in September, 2010 in the amount of \$92,775 and its fourth allocation from the Prostate Cancer Research State Income Tax Check-Off Program in the amount of \$35,145. Applications for new Breast and Prostate Cancer Research Projects were solicited in the winter of 2010 and the spring of 2011.

C. RESEARCH PROJECTS SELECTION PROCESS

At the Medical College of Wisconsin, breast and prostate cancer related research grant and fellowship applications are selected using a peer-review process that draws upon both standing and ad hoc Cancer Center Research Grant Committees. MCW faculty are selected for service on these committees to provide wide representation from the basic science departments, clinical departments and the disciplines involved in cancer related research within the College.

The complexity of the projects submitted seeking funding from the Breast and Prostate Cancer Research State Tax Check-Off programs warranted a highly technical review. The review panel consisted of basic and translational scientists, clinicians, and bio-statisticians. Because of the highly technical and basic science nature of the proposals, a lay-person was not included on the review panel.

Research grant applications were reviewed in the winter of 2010 by the Cancer Center's Research Grants Committee. Postdoctoral fellowship applications were reviewed in the spring of 2011 by an ad hoc review committee. The applications were prioritized on the basis of scientific merit, and the recommendations for funding were submitted to the Cancer Center's Senior Leadership Committee. The Senior Leadership Committee of the Cancer Center is responsible to the Dean of the Medical College for appropriate allocation of funds from the State of Wisconsin Breast and Prostate Cancer Research State Income Tax Check-Off programs. The final decisions on awards were made after ensuring that the recommended applications complied with State requirements that funding be used for breast or prostate cancer research only and that the funding is non-supplanting. The Principal Investigator must sign a non-supplanting attestation form.

Three awards were made in the past year: One two-year research grant in prostate cancer (\$100,000) was awarded to Dr. Kathryn Bylow, MD, Assistant Professor, Department of Medicine, MCW; and two one-year

interdisciplinary postdoctoral fellowship research project grants in breast cancer (\$50,000 each) were made to: (a) Howard Jacob, PhD (Department of Physiology, MCW) and Michael Dwinell, PhD (Department of Microbiology and Molecular Genetics, MCW) and to (b) Carol Williams, PhD (Department of Pharmacology, MCW), Julia White, MD (Department of Radiation Oncology, MCW) and Alexander Mackinnon, MD, PhD (Department of Pathology, MCW). These research projects are described in Section D below.

The residual funds in the State of Wisconsin Tax Check-Off Program in Breast and Prostate Cancer Research will be carried over and combined with the 2011 appropriations (from FY10) and used in 2012 to fund additional breast and prostate cancer research projects.

D. BREAST AND PROSTATE CANCER RESEARCH PROJECTS FUNDED IN FY2010-2011

<u>Title of Research Project:</u>	“Use of β -hydroxy- β -methylbutyrate (HMB) to Counteract Loss of Muscle Mass and Strength in Older Men with Recurrent Prostate Cancer Started on Androgen Deprivation Therapy (ADT)”
<u>Principal Investigator:</u>	Kathryn Bylow, MD, Assistant Professor, Department of Medicine, Medical College of Wisconsin
<u>Co-Investigators:</u>	James Thomas, MD (Dept. of Medicine, MCW), Alex Ng, PhD (Marquette University), Danny A. Riley, PhD (Dept. of Cell Biology, MCW), and Robert Prost, PhD (Dept. of Radiology, MCW)
<u>Amount Awarded:</u>	\$100,000
<u>Period of Award:</u>	Two years (July 2011 – June 2013)

Lay Description of Research Project (provided by the Principal Investigator):

APPROACH: The standard treatment for recurrence of prostate cancer is androgen-deprivation therapy or ADT. It is well established that ADT causes muscle weakness and wasting, osteoporosis, and fatigue. In a preliminary study, the applicant has found that older men on ADT have significant physical performance deficits, functional impairments, and falls at a higher rate than men in the general population. Thus, ADT may be causing significant toxicity to some patients with prostate cancer.

Hydroxy-methylbutyrate (HMB) is a metabolite of the amino acid leucine and has been shown to decrease the rate of muscle breakdown. When given with amino acid supplementation (arginine and lysine or glutamine), HMB improves strength, fat-free mass and function in the general geriatric population who are prone to loss of muscle mass and strength (sarcopenia). The use of HMB with amino acid supplementation in older men with prostate cancer and receiving androgen-deprivation therapy has not been reported. The applicants HMB plus arginine and glutamate (AG) will reduce the adverse change in body composition and strength/muscle fatigability that occur when older men with prostate cancer are started on ADT. To test this hypothesis, the applicants propose to conduct a prospective cohort pilot study of men age 65 and older with recurrent prostate cancer who are about to start ADT. The men will be randomized in a double blind fashion to receive HMB + AG versus placebo. Double blind” means that neither the patient(s) nor their doctor(s) will know whether the patient is receiving the drugs or the placebo (inactive substance). The men will be followed for 3 months from start of ADT. With data obtained in this study, a larger randomized study will be designed to compare interventions aimed at minimizing the adverse effects that occur with ADT initiation.

The specific aims of the study are:

- (1) To prospectively measure the change in body composition, strength, physical performance measures in blinded cohorts (i.e., those receiving HMB + AG and those receiving placebo);
- (2) To prospectively measure the fatigability and muscle size using MRI and MRI spectroscopy in the blinded cohorts; and
- (3) To prospectively analyze muscle fiber cross-sectional area and muscle fiber type using muscle biopsies taken from a subset of men in both cohorts.

SIGNIFICANCE: This is a clinically important but relatively understudied area that has potential for improving the patients quality of life. The study is novel. Use of nutritional supplementation with HMB + AG in men with recurrent prostate cancer has not been reported previously.

INVESTIGATORS: This study involves a multi-disciplinary team including a geriatrician (Kathryn Bylow, MD), medical oncologist (James Thomas, MD, PhD), radiologist (Robert Prost, PhD), pathologist (Danny Riely, PhD) and exercise physiologist (Alexander Ng, PhD). The Principal Investigators, Dr. Bylow, is an Assistant Professor in the Department of Medicine at MCW and specializes in geriatric medicine.

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<u>Title of Research Project:</u>	“Characterizing Genetic Risks of Metastasis using Breast Cancer Xenografts in Immunodeficient Consomic and Congenic Rat Strains”
<u>Principal Investigator:</u>	Howard Jacob, PhD, Professor, Department of Physiology, Medical College of Wisconsin
<u>Co-Investigator:</u>	Michael Dwinell, PhD (Dept. of Microbiology, MCW)
<u>Post-Doctoral Fellow:</u>	Michael J. Flister, PhD
<u>Amount Awarded:</u>	\$50,000
<u>Period of Award:</u>	One year (July 2011 – June 2012)

Lay Description of Research Project (Adapted from that provided by the Principal Investigator):

Significance: In 2010, breast cancer was the most prevalent malignancy in women and accounted for 15% of all female cancer-related deaths (ACS Annual Report). Familial inheritance is associated with 25-30% of breast cancer patients, demonstrating that in many cases there are strong genetic risks associated with breast cancer. However, of these cases, only 3-8% have been linked to causative genes¹ and even fewer polymorphisms have been directly associated with metastasis, the primary cause of cancer mortality. The objective of this project is to fill the significant knowledge gap by characterizing genetic variants that mediate risk of breast cancer metastasis that will impact patient survival. To achieve this objective, we are proposing a new model of human breast cancer that combines conventional breast cancer xenografts with immunodeficient consomic and congenic rat strains (i.e., isogenic background except for one substituted chromosome). Rather than focusing solely on genetic and epigenetic changes in malignant cells (i.e., the seeds), this approach characterizes genomic differences in the host environment (i.e., the soil), which also plays a central role in cancer metastasis. Our hypothesis that host genome will affect metastasis is based on previous findings that single chromosomes from the Brown Norway (BN) strain significantly decrease mammary carcinogenesis when introgressed onto the tumor-susceptible Dahl salt-sensitive (SS) background. This will complement the current understanding of the genomic abnormalities underlying malignancy and provide a more complete picture of susceptibility to breast cancer metastasis, while at the same time characterizing novel targets for development of more efficacious anti-metastatic treatments.

Specific Aims: To genetically map genes that mediate susceptibility or resistance to breast cancer metastasis and progression using spontaneously metastatic (e.g., MDA-MB-231Luc and MDA-MB-435Luc) and poorly-

metastatic (MCF-7Luc) human breast cancer xenografts in immunodeficient consomic and congenic rat strains. Including analyses of both aggressive and non-aggressive breast cancer lines will give us the flexibility to identify genetic variants that increase or decrease breast cancer metastasis. A future outcome of this project is to screen the full SS-BN consomic panel (22 strains) for susceptibility to metastasis. However, due to the scope of this proposal, we will limit our initial analyses to protected (consomic strains SSBN) and susceptible (SSBN10) rat strains that have shown the greatest differences in response to mammary carcinogenesis in our laboratory and other previously reported models. Moreover, successful analysis of BN6 and BN10 chromosomes will be aided by multiple congenic strains that have been previously generated to narrow protective regions within the chromosomes 6 and 10. By comparing tumor progression and metastasis in experimental and parental strains, we will rapidly identify loci within chromosomes 6 and 10 that affect breast cancer metastasis.



<u>Title of Research Project:</u>	“The Prognostic Significance of SmgGDS-mediated Signaling Pathways in Breast Cancer”
<u>Principal Investigator:</u>	Carol Williams, PhD, Associate Professor, Department of Pharmacology, Medical College of Wisconsin
<u>Co-Investigator:</u>	Julia White, MD (Dept. of Radiation Oncology, MCW) and Alexander Mackinnon, MD, PhD (Dept. of Pathology, MCW)
<u>Post-Doctoral Fellow:</u>	Carmen Bergom, MD, PhD
<u>Amount Awarded:</u>	\$50,000
<u>Period of Award:</u>	One year (July 2011 – June 2012)

Lay Description of Research Project (Adapted from that provided by the Principal Investigator):

Advances in understanding the molecular basis of breast cancer progression and resistance has led to new treatments, such as HER2 and estrogen signaling inhibition. However, last year over 200,000 new cases of breast cancer were diagnosed, and over 40,000 breast cancer-associated deaths occurred. Small GTPase enzymes regulate breast cancer development and progression and can alter chemotherapy and radiation sensitivity. To promote these functions, small GTPases must be chemically altered by the addition of a C-terminal isoprenyl group, which anchors and localizes small GTPases to cell membranes. We recently discovered that two splice variants of SmgGDS, SmgGDS-588 and SmgGDS-607, regulate small GTPase prenylation and membrane localization. Our studies demonstrate that SmgGDS is upregulated in breast cancer as well as lung and prostate cancer. SmgGDS levels also increase in doxorubicin-treated breast cancer cells, and reduction of SmgGDS-558 or SmgGDS-607 levels augments doxorubicin-mediated breast cancer cell death (prelim. data). In addition, murine SmgGDS^{-/-} thymocytes are more sensitive to etoposide and UV irradiation⁴, and reduction of SmgGDS splice variants dramatically reduces anchorage-independent colony formation and proliferation of breast cancer cells (prelimin. data). Furthermore, SmgGDS (gene name RAP1GDS1) is one of a number of mRNAs that, when increased, decreased the likelihood of complete pathologic response to neoadjuvant breast cancer chemotherapy. Taken together, these data suggest that SmgGDS is a promising target to predict and potentially modulate breast cancer progression.

Our long term goal is to understand how SmgGDS splice variants can be manipulated to enhance breast cancer treatment. Our overall hypothesis is that SmgGDS splice variants are overexpressed in human breast cancer, causing increased small GTPase prenylation, NF- κ B activation, and COX-2 expression, with levels of SmgGDS ultimately correlating with increased likelihood of breast cancer recurrence. Here at MCW, we have all the tools to test this hypothesis in vitro, in vivo, and in human breast tumors. This multidisciplinary

project has the potential to identify new prognostic indicators for breast cancer and uncover new therapeutic targets. The following specific aims will help elucidate the role of SmgGDS in human breast cancer progression:

Aim 1: Determine whether proteins levels of SmgGDS, its splice variants, and its putative downstream effectors Rap, Rac, and COX-2 correlate with breast cancer molecular subtype and progression.

Aim 2: Determine whether SmgGDS splice variants mediate overexpression of COX-2 and activation of the small GTPases Rap1 and Rac1 in human breast tumor xenografts.

Taken together, these Specific Aims will determine whether expression of SmgGDS isoforms is predictive and/or prognostic of a poorer outcome from human breast cancer, and examine which downstream mediators of SmgGDS may promote breast cancer progression. Using a multidisciplinary approach, we can synthesize clinical and molecular data in a large sample of human breast tumors via tissue microarray (TMA) technology and examine the correlates of these findings in murine xenograft models, providing truly translational research findings. The TMA created for this project will also be useful for future collaborations with a variety of investigators who are interested in examining whether other signaling pathways correlate with breast cancer prognosis. In summary, the basic and translational components of this hypothesis have the potential to further investigations of novel mediators of breast cancer progression in vivo.

E. IMPLEMENTATION OF RESEARCH PROJECTS

Project: "Use of β -hydroxy- β -methylbutyrate (HMB) to Counteract Loss of Muscle Mass and Strength in Older Men with Recurrent Prostate Cancer Started on Androgen Deprivation Therapy (ADT)" (PI: Kathryn Bylow, MD): The research project selection process was completed in the early part of 2011, and the funds were administratively committed to the Principal Investigators in April 2011. As required for protection of human subjects, the clinical protocol for this project was submitted to the Institutional Review Board (IRB) for review and approval. Because of revisions requested by the IRB, final approval of the protocol is still pending at the time of this report. Accrual of patients onto the protocol is anticipated to begin early in 2012. An update on the status and a progress report will be provided in next year's annual report.

Project: "Characterizing Genetic Risks of Metastasis using Breast Cancer Xenografts in Immunodeficient Consomic and Congenic Rat Strains" (Howard Jacob, PhD): The research project selection process was completed in the spring of 2011, and funds were administratively committed to the Principal Investigator, Dr. Jacob, in April 2011. Work on the project commenced after documentation of compliance with relevant state, federal and institutional regulations was provided. A progress report will be included with next year's annual report.

Project: "The Prognostic Significance of SmgGDS-mediated Signaling Pathways in Breast Cancer" (PI: Carol Williams, PhD): The funds were administratively committed to the project in April 2011. Work on the project commenced in July 2011 after documentation of compliance with any and all relevant state, federal and institutional regulations was provided. Work on the research project has been underway for approximately 6 months. Breast tumor tissue micro-arrays are being generated, and experiments testing the growth of breast tumor xenografts in mice have begun. These research accomplishments and additional findings will be described in the First-Year Progress Report, to be provided in next year's annual report.

F. PROGRESS REPORTS FROM PROJECTS AWARDED IN THE PREVIOUS TWO YEARS

Nine research grants relevant to breast and/or prostate cancer have been awarded since the inception of the State of Wisconsin Breast and Prostate Cancer Research State Income Tax Check-Off Programs. We track the progress and scholarly output of grants for two years after their completion. The grants funded in the last two fiscal years are listed below, and an updated progress report for each is appended to this report (see Appendix). The grants are:

Title of Research Project: "Disruption of Mitochondrial Thiol Redox State by Triapine: A Novel Anti-Cancer Mechanism"
Principal Investigator: Charles R. Myers, PhD, Professor, Department of Pharmacology & Toxicology, Medical College of Wisconsin
Amount Awarded: \$100,000
Period of Award: Two years (July 2010 – June 2011)



Title of Research Project: "Nogo-B Receptor Modulates Sensitivity of Breast Cancer Cells to Chemotherapy and Radiation Therapy"
Principal Investigator: Qing (Robert) Miao, PhD, Assistant Professor, Department of Surgery (Pediatric Surgery), Medical College of Wisconsin
Amount Awarded: \$133,333
Period of Award: Two years (June 2009 – May 2011)

G. REPORT SUMMARY

Research into the fundamental biology of breast and prostate cancer holds the promise of identifying new approaches to the treatment or prevention of the cancers and the Medical College of Wisconsin has invested the funds provided by Wisconsin taxpayers in new pilot research projects.

These projects undergo a multi-level process of peer-review to assess their scientific merit and significance to breast and prostate cancer. During academic year 2010-2011, three awards totaling \$200,000 were made: One to support a promising new clinical research project on prostate cancer, and two supporting innovative new interdisciplinary research projects on breast cancer.

Residual funds from the Breast and Prostate Cancer Research State Income Tax Check-Off Programs will be carried over into the next year and combined with the next appropriation from FY10 to fund additional research projects in FY12.

We have previously reported on the nine research projects funded since 2007 with allocations from the Breast and/or Prostate Cancer Research State Income Tax Check-Off Programs. In this annual report, we provide information on (a) three new projects funded in FY11 and (b) progress reports on the two projects funded during the past two academic years (FY09 and FY10).



**MEDICAL COLLEGE OF WISCONSIN
DECEMBER 29, 2011**

APPENDIX - PROGRESS REPORTS

**BREAST AND PROSTATE CANCER RESEARCH STATE INCOME TAX CHECK-OFF PROGRAM
REPORTING PERIOD JULY 1, 2010 - JUNE 30, 2011
MADE IN THE LAST TWO ACADEMIC YEARS (FY09 AND FY10)**

Title of Research Grant: "Disruption of Mitochondrial Thiol Redox State by Triapine: A Novel Anti-Cancer Mechanism"
Principal Investigator: Charles R. Myers, PhD, Professor, Department of Pharmacology & Toxicology, Medical College of Wisconsin
Amount Awarded: \$100,000
Period of Award: Two years (May 2010 – April 2012)

Title of Research Grant: "Nogo-B Receptor Modulates Sensitivity of Breast Cancer Cells to Chemotherapy and Radiation Therapy"
Principal Investigator: Qing (Robert) Miao, PhD, Assistant Professor, Department of Surgery (Pediatric Surgery), Medical College of Wisconsin
Amount Awarded: \$133,333
Period of Award: Two years (June 2009 – May 2011)

State of Wisconsin Income Tax Check-Off Program for
Breast & Prostate Cancer Research - 2011
FIRST YEAR PROGRESS REPORT

Amount \$100,000 Term: from May 1, 2010 to April 30, 2012

Investigator (Name, Title, Department) and Co-Investigators (if any):

Charles R. Myers, PhD, Professor, Department of Pharmacology

Project Title:

"Disruption of Mitochondrial Thiol Redox State by Triapine: A Novel Anti-Cancer Mechanism"

Results (State the hypothesis and specific aims and briefly summarize work to date):

Hypothesis and Specific Aims:

The overall hypothesis is that the disruption of mitochondrial thiol redox balance is an important component of the cytotoxic effects of the anti-cancer drug triapine. The specific aims will test selected aspects of the hypothesis:

1. Determine if the mitochondrial proteins TrxR2
2. Determine if mitochondria accumulate iron-triapine, which enhances mitochondrial ROS generation. The resulting pro-oxidant effects are important for cytotoxicity.

Progress to Date:

Triapine (Tp) is an iron chelator with activity against several types of cancer. It may have multiple mechanisms of action that are not well understood. Prior evidence suggested that iron-Tp $[Fe(Tp)_2]$ is the cytotoxic form and that its redox cycling can generate reactive oxygen species. Prior to our work, however, there was no evidence that Tp causes redox stress in cells. Using thioredoxins (Trx) and peroxiredoxins (Prx) as cellular indicators of redox stress, we showed that Tp causes pronounced oxidation of the mitochondrial forms (Trx2 and Prx3), with little effect on cytosolic Trx1. Thus, Tp causes pronounced redox stress that is largely directed at the mitochondria. These effects are dependent on both the dose and duration of exposure to Tp. The human breast cancer cell lines MCF7 and MDA-MB-231 are more sensitive to Tp than the normal breast epithelial line MCF-10A. This is reflected in the greater sensitivity of Prx3 to oxidation in the breast cancer cells.

The Trx2/Prx3 oxidation did not require the inhibition of total thioredoxin reductase (TrxR), although effects on the cytosolic vs. mitochondrial isoforms remain to be determined. For some drug treatments, Prx3 was oxidized before Trx2, implying that Tp may promote mitochondrial peroxide generation. The generation of reactive oxygen species (ROS) likely results from the redox cycling of iron-Tp, but the cellular mechanisms are not understood. Cysteine (Cys) is a faster and more efficient reductant of $Fe(III)(Tp)_2$ than is reduced glutathione (GSH). Cys also generates more ROS from $Fe(III)(Tp)_2$ than does GSH. However, there is not much free Cys in cells, so Tp redox cycling may well be enzymatic. We determined that TrxR reduces $Fe(III)(Tp)_2$ by a mechanism that involves its flavin and the C59 residue, but not its active site selenocysteine (SeCys). This process consumes O_2 , and ESR spin trapping shows strong signals for hydroxyl radical (HO^\bullet) and a carbon radical. Some superoxide ($O_2^{\bullet-}$) is also detected. While TrxR can reduce the $O_2^{\bullet-}$ adduct to the HO^\bullet adduct, the SeCys-minus and C59S variants of TrxR cannot, yet they still generate HO^\bullet when incubated with $Fe(Tp)_2$, verifying that HO^\bullet is in fact generated. Catalase largely eliminates the HO^\bullet signal indicating that it is peroxide-dependent, and not due to the

reduction of the $O_2^{\bullet-}$ adduct.

Findings to date indicate that the carbon radical involves iron-Tp, and not just Tp. While the carbon radical becomes prominent when $Fe(III)(Tp)_2$ is increased from 25 to 50 μM , adding 100 μM Tp to 25 μM $Fe(III)(Tp)_2$ does not enhance the carbon radical. The carbon radical is peroxide-dependent, i.e. it is eliminated by catalase, but not by superoxide dismutase which should enhance peroxide generation. We predict that peroxide plus $Fe(II)(Tp)_2$ are required to generate this carbon radical.

The iron-Tp interaction with TrxR is somehow special, i.e. it does not reflect findings with other Fe chelates. TrxR showed little to no ability to reduce $Fe(III)EDTA$, and is notably less efficient at reducing $Fe(III)(Dp44mT)_2$ than $Fe(III)(Tp)_2$ (Dp44mT is another tridentate Fe chelator). Consistent with this, $Fe(III)(Dp44mT)_2$ results in considerably smaller HO^\bullet and $O_2^{\bullet-}$ signals, and little to no carbon radical. Adding SOD largely eliminated the HO^\bullet and $O_2^{\bullet-}$ signals from $Fe(Dp44mT)_2$ indicating $O_2^{\bullet-}$ -dependence of both signals. Catalase had a limited effect on the HO^\bullet signals, indicating that the HO^\bullet adduct largely resulted from reduction of the $O_2^{\bullet-}$ adduct. The $Fe(Tp)_2$ results cannot be explained by some residual unchelated Fe, as we get the same results when Fe-Tp is prepared with three equivalents of Tp as we do with two equivalents.

Overall, the data indicate that Tp causes pronounced mitochondrial redox stress that could involve various reactive species, including HO^\bullet , H_2O_2 , and iron-Tp radicals. There is a central role of peroxide in these events which could explain the particular sensitivity of Prx3. Several observations indicate that this may be an important mechanism by which Tp kills cancer cells:

- breast cancer cells are more sensitive to Tp than are normal breast cells, and this correlates with the relative Prx3 sensitivity in these cells;
- cancer cells are generally known to be more peroxide sensitive than normal cells;
- cancer cells are often particularly sensitive to mitochondrial redox stress;
- cancer cells typically overexpress the transferrin receptor which enhances iron uptake and therefore the potential to form $Fe(Tp)_2$;
- cancer cells often overexpress TrxR which could enhance the redox cycling of $Fe(Tp)_2$.

Given the important role of mitochondria in controlling cell death, and the ability of mitochondrial oxidant stress to induce cell death, these previously unrecognized effects of Tp could contribute to its overall cytotoxic effects and therefore lead to a better understanding of its therapeutic potential and possible synergism with other anti-cancer strategies. Future experiments will: (1) further explore the mechanisms of peroxide generation and its role in cell death, (2) determine if peroxide-generating strategies can enhance the effectiveness of Tp, and (3) examine the uptake of $Fe(Tp)_2$ by mitochondria.

List any publications since the grant was awarded (published, in press or submitted):

J. M. Myers, W. E. Antholine, J. Zielonka, C. R. Myers. 2011. The iron-chelating drug triapine causes pronounced mitochondrial thiol redox stress. *Toxicol. Lett.* 201:130–136.

List any abstracts, poster or presentations, papers, and other scholarly works since the grant was awarded:

C.R. Myers, W.E. Antholine, J.M. Myers. 2010. The iron-chelating antitumor agent triapine causes pronounced thiol redox stress in the mitochondria of human cells. *Free Radic. Biol. Med.* 49 Suppl. 1, abstract 471, p. S170. Presented at the SFRBM annual meeting, November, 2010.

List any national grants submitted, planned, or awarded since start-up of the grant

None submitted to date. Additional data will be obtained before submissions.

List any other grants or awards you have obtained:

The following 1-yr bridge funding was obtained from NIH to obtain additional data to address the reviews for an R01 renewal: 2R56ES012707-05A1, Human Lung Chromium Toxicity: Oxidant Stress. P.I.: Myers. May 1, 2010–April 30, 2012. Direct Funds:\$150,000.

The following 1-yr award is from the MCW Innovation Center for mass spectrometry method development: "Mass spectrometry method development for aldehyde adducts on proteins". P.I.: Myers. Robert D. and Patricia E. Kern Family Foundation. Aug. 2011–Aug. 2012. Direct Funds: \$5,000.

List any patents granted or applied for: None.

**State of Wisconsin Income Tax Check-Off Program for
 Breast & Prostate Cancer Research - 2011
 SECOND YEAR PROGRESS REPORT**

Amount	<u> \$133,333 </u>	Term: from	<u> June 2009 </u>	to	<u> May 2011 </u>
Investigator (Name, Title, Department) and Co-Investigators (if any):					
Qing (Robert) Miao, PhD, Department of Surgery (Pediatric Surgery), Medical College of Wisconsin					
Project Title:					
"Nogo-B Receptor Modulates Sensitivity of Breast Cancer Cells to Chemotherapy and Radiation Therapy"					
Results (Briefly describe work to date):					
<p>In 2010 and 2011, we made three major progresses to our proposed studies. <i>First</i>, we performed in vivo tumor xenograft in nude mice. To avoid the adversely effects of NgBR knockdown on established cell line proliferation and cell survival, we took the advantage of the TRIPZ lentiviral Tet-On inducible shRNA from OpenBiosystem that allows to knock down NgBR by inducing shRNAi expression with doxycycline. We established MDA-MB-231 cell lines that can regulate NgBR expression by doxycycline administration. NgBR expression decreased in the presence of doxycycline for 48 hours as compared to that in the absence of doxycycline, and then EGF-stimulated phosphorylation of Akt also decreased in cells treated with doxycycline due to NgBR knockdown. NgBR expression and EGF-stimulated phosphorylation of Akt recovered after the removal of doxycycline for 48 hours. It provides us a powerful tool to temporally regulate NgBR expression and NgBR-mediated EGF signaling that is involved in tumor cell survival and tumor cell resistance. We injected these engineered breast cancer cells subcutaneously into the back area of nude mice. When tumor size reached 200 mm³, mice were divided into two groups, one group was fed with doxycycline and another without doxycycline as control group. The results showed that inducible knockdown of NgBR retarded tumor growth by 33% during 3 week experiment course. It demonstrated that doxycycline-inducible system works well in vivo and NgBR is a potential therapeutic target for breast cancer. We are performing histology analysis of tumor sections. We are establishing collaboration with breast cancer clinical team at the Department of Surgery, MCW. As suggested by breast cancer surgeons, we are testing the synergistic effects of NgBR knockdown and chemotherapeutic drugs on breast tumor cell growth and survival in vitro. We found NgBR knockdown can increase the sensitivity of several chemotherapeutic drugs that are currently applied in breast cancer treatment, such as paclitaxel, docetaxel, doxorubicin and 5-Fluorouracil. In the next step, we will test the synergistic effects of</p>					

NgBR knockdown and these chemotherapeutic drugs on tumor growth in vivo. These finding should have huge impact to breast cancer therapy. *Second*, we are futher examining the molecular mechanisms by which NgBR coordinats with EGF receptors (EGFRs) to acivate Ras. Our preliminary results showed that NgBR knockdown impaired the EGF-stimulated Ras activation and consequent phosphorylation of Akt and ERK in MDA-MB-231 breast cancer cells. Based on these results, we proposed a working model that NgBR binds farnesylated Ras and increases membrnae-associated Ras, and EGFR activation recruits Ras activator, SOS, to cell plasma membrane for activating Ras recruited by NgBR. To prove this hypothesis, we isolated membrane fraction by ultracentrifugation approach and examined SOS as well as Ras in the membrane fractions. The results show that Nogo-B (a ligand for NgBR) stimualtion increases the membrane-associated SOS and Ras, but NgBR knockdown remarkedly decreases the accumulation of membrane-associated SOS and Ras in MDA-MB-231 cells. These results further demonstrated that NgBR overexpression in breast cancer cells promotes EGF signaling by increasing the membrane-associate Ras. It suggests that NgBR is a potential therapeutic target for Ras-denpedent cancer. *Third*, we successgully identified the critical residues in the cytoplasmic domain of NgBR that are essential for binding farnesylated Ras and activation of Ras signaling. This finding will provide very helpful information to design small molecule drug to block the binding of NgBR and Ras.

List any publications since the grant was awarded (published, in press or submitted):

We have one manuscript that has been published in Blood, which demonstrated the role of NgBR in regulating in vivo angiogenesis during zebrafish development. We are working on our second manuscript about the role of NgBR in regulating Ras translocation and breast tumor growth.

List any abstracts, poster or oral presentations, chapters, and other scholarly works since the grant was awarded:

1. Roles of NgBR in promoting Ras translocation and Ras signaling in human breast cancer-cells. Second AACR International Conference on Frontiers in Basic Cancer Research, San Francisco, CA, 2011.
2. Nogo-B receptor (NgBR) is a new therapeutic target for increasing the sensitivity of breast cancer to chemotherapy and irradiation. Tumor Microenvironment Complexity: Emerging Roles in Cancer Therapy, Orlando, FL, 2011.
3. NgBR is a potential therapeutic target for resistant breast cancer” Poster presentation in First Annual Fall Research Seminar at the Department of Surgery, Medical College of Wisconsin, October 22, 2010

List any national grants submitted, planned, or awarded since start-up of the grant:

We submitted an application for the Idea Award to Department of Defense Breast Cancer Research Program based on the continuous development from the currently funded research proposal. Although we received a score of 1.9 (excellent category), our application is not recommended for funding because the percentile for funding is 4%. We will revise our previous application and resubmit to Department of Defense Breast Cancer Research Program in 2012. We also plan to develop this proposed studies to NCI R01 grant in 2012. In 2011, we received an NIH R01 grant award to study the role of NgBR in regulating Ras translocation and Ras activation in endothelial cells and its regulatory roles in endothelial cell function.

List any other grants or awards you have obtained:

Active

Title: Roles of Nogo-B and Nogo-B receptor (NgBR) in tumor angiogenesis
Source: American Cancer Society Pilot Research Grant
Role: Principal Investigator
Dates: 7/2011 - 6/2013

Title: Role of NgBR in endothelial cell function and angiogenesis
Source: NIH/NHLBI R01 HL108938
Role: Principal Investigator
Dates: 9/1/2011 - 7/31/2016

Completed

Title: Role of Nogo-B receptor in regulating endothelial cell functions
Source: American Heart Association
Role: Principal Investigator
Dates: 1/1/2007 - 6/30/2011

List any patents granted or applied for:

None to date



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December 12, 2011

Honorable Scott Walker
Governor, State of Wisconsin
Room 115 East, State Capitol
Madison, WI 53702

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Dear Governor Walker:

Enclosed please find the Medical College of Wisconsin's (MCW) annual report for the *Breast and Prostate Cancer Research Program*. This Program is funded through the Wisconsin income tax check-off created by the 2003 Wisconsin ACT 176 (breast cancer) and the 2005 Wisconsin ACT 460 (prostate cancer). The law requires that MCW use this money for breast and prostate cancer research and to report annually on the use of funds.

This document reports activities, projects and research funded through the *Breast and Prostate Cancer Research Program* at MCW for the period of **July 1, 2010 – June 30, 2011**.

If you have any questions or comments regarding this report, please contact me at 414.955.8217.

Sincerely,

Kathryn A. Kuhn
Vice President, Government and Community Relations
Medical College of Wisconsin

Enclosure:

Medical College of Wisconsin 2011 Breast and Prostate Cancer Research Program Report

Cc:

- John R. Raymond, Sr., MD, *President and Chief Executive Officer, Medical College of Wisconsin*
- Joseph E. Kerschner, MD, *Dean and Executive Vice President*
- Ming You, MD, PhD, *Professor and Director, Cancer Center, Medical College of Wisconsin*
- Robert Truitt, *Emeritus Professor, Medical College of Wisconsin*
- Robert J. Marchant, *Senate Chief Clerk*
- Patrick E. Fuller, *Assembly Chief Clerk*
- Emily Pope, *Legislative Fiscal Bureau*
- Sara Hynek, *Department of Administration*

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