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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Scientific Training.....	7
Conclusion and Future Research Directions.....	7
References.....	8
Appendices.....	9
Supporting Data.....	16

INTRODUCTION: Obesity increases risk for breast cancer in postmenopausal women and increases mortality in pre- and postmenopausal women, in fact, 30-50% of breast cancer deaths in post-menopausal women may be attributed to excess body weight (1). The HER-2/neu proto-oncogene is amplified in 25-30% of human primary breast cancers, and increased levels of HER-2/neu expression in tumors can have a negative impact on prognosis of the cancer (2). Approximately two-thirds of breast cancers arising in postmenopausal women are positive for estrogen receptor (ER) in carcinoma cells. However, tumors negative for estrogen receptor (ER-) confer a much worse prognosis (3). Energy balance modulation through diet-induced obesity and calorie restriction has been shown to modulate serum levels of many growth factors and hormones, including estrogen. Additionally, energy balance modulation affects cancer initiation and progression multiple mouse and primate models (4). However, the specific mechanisms by which obesity affects ER- breast cancer risk or prognosis are not clearly understood, and strategies for offsetting the negative effects of obesity are urgently needed, so the purpose of my project is twofold. First, we will determine which obesity-related growth factors/hormones are key to tumor progression. Second, we will determine how blocking specific growth factors (specifically components of the IGF-I pathway) can decrease the negative effects of obesity on breast cancer and increase response to chemopreventive drugs (chemopreventive drugs are compounds given to *prevent* breast cancer, i.e. tamoxifen). This annual report summarizes the characterization of the effects of dietary energy balance modulation on metabolic hormones and mammary tumor development, growth, and progression in MMTV-neu (HER-2/neu overexpressing) mice (**Specific Aim 1**).

We have chosen the MMTV-neu transgenic mouse model of spontaneous mammary tumorigenesis because the course of breast tumor development in these mice is similar to the tumorigenesis process in many women. Mammary tumors proceed from hyperplasias (which are ER-alpha-positive) to ductal carcinomas in situ (which are predominantly ER-negative) to invasive adenocarcinomas (which are alpha-negative and develop in nearly 100% of the non-obese mice by 10 ER- months of age. This model has been frequently used for chemoprevention studies. While the impact of obesity has not been reported in this model, the mTOR inhibitor rapamycin was shown to have inhibitory activity in a therapeutic setting in these mice (5). As mTOR is downstream of IGF-1, this data demonstrates the likely role of IGF-I in HER2/neu-driven mammary tumors, and supports the plausibility of our hypothesized interactions between energy balance, IGF-I receptor activation and breast cancer. The rationale for using mice in this study is that the effects of tumor response to therapeutic agents cannot be modeled in simpler systems, and all the variables proposed in this study cannot be effectively controlled in humans. Simpler systems cannot effectively model obesity; therefore, we must use mice. Additionally, IGF-I inhibitors are not yet FDA approved as a chemopreventive agent in humans.

BODY: In our original proposal, we hypothesized that: ***Obesity-induced increases in circulating IGF-1 levels promote IGF-1/ER crosstalk in the mammary epithelium, leading to a reduction in the chemopreventive efficacy of tamoxifen.*** In Specific Aim 1, we outlined the **characterization the effects of dietary energy balance modulation on metabolic hormones and mammary tumor development, growth, and progression in MMTV-neu mice.** The model in which we have chosen to test this hypothesis is the MMTV-neu transgenic mouse model, in which mice were fed a standard diet-induced obesity regimen. **Task 1** was to modulate diets in MMTV-neu mice and measure effects on tumor. The first **Milestone** was to receive IACUC approval,

which was accomplished on 13 August 2008, renewed on 06 July 2009, on 03 August 2010, and on 11 July 2011, and as needed for personnel and minor modifications. The USAMRMC has been notified of all major and minor protocol modifications. **Task 1.a** was accomplished when MMTV-neu mice were ordered and put on diet regimens for 90 weeks. 180 female MMTV-neu transgenic mice, and 180 female, age-matched, wild-type (FVB) mice were used for this study. Half of the MMTV-neu (90 mice) and half of the wild-type mice (90 mice) were ovariectomized to mimic the post-menopausal state. Mice were placed on one of the following diets: High-fat diet-induced obesity diet, Control ad-libitum diet, or 30% caloric restriction diet (iso-nutrient, but with 30% reduction in calories from the Control fed mice). We had 12 experimental groups, each containing 30 mice. A subset of 4 mice per group were sacrificed at the following timepoints: Baseline (date of arrival of mice, 4-6 weeks old, never exposed to diet; 48 mice), 2 months old (48 mice), 4 months old (48 mice), and 6 months old (48 mice). These mice were non-tumor bearing, and normal tissues were collected, including serum and mammary fat pad in order to determine the effect of energy balance on circulating serum hormones (including IGF-I) on the expression of ER α/β during the development of hyperplasia, DCIS, and resulting ductal adenocarcinoma in these mice. The number of animals required for this experiment was determined using the power analysis spreadsheet downloaded from http://www.hms.harvard.edu/orsp/coms/Statistics/Statistical_Explanation_from_Dr._LaMorte_of_BU.htm. Using 90% power to detect a 20% difference between the groups with an alpha level equal to 0.05, we determined that a minimum sample size of 15 mice was required to detect significant differences with respect body composition and hormonal profiles. Therefore, to be certain that we are able to detect differences, we determined that approximately 15 mice per experimental group would be optimal (after all baseline, 2 month, 4 month, and 6 month sacrifices were completed). Our collaborator, Mr. Kevin Lin, a biostatistician in the Biometry Research Group at UT-MD Anderson Cancer Center, assisted in the design and statistical analysis of the study. All mice were palpated 2x/week. Once a palpable tumor developed, tumor growth was measured twice per week with electronic calipers, and tumor volume will be calculated as $(W*W*L)/2$ to give you cm^3 . When tumors reached 1.5cm in diameter, animals were sacrificed, and tumor weight measurements were taken (**Task 1.c**). Other tissues were harvested from the mice at the time of sacrifice, including tumor, mammary fat pad, liver, visceral white adipose tissue, skin, and skeletal muscle (**Task 1.d**). All mice still alive after 90 weeks of diet were killed, mammary fat pads were harvested for whole mount analysis, and other listed tissues were harvested. Blood samples were taken for fasting glucose measurements and for serum hormone analysis at the time of sacrifice by cardiac puncture on all animals both normal and tumor-bearing (**Task 1.b**). Tumor and normal mammary tissue was collected; half of the tumor and normal tissue was fixed in formalin overnight and then embedded in paraffin, while the other half was flash frozen in liquid nitrogen for signaling pathway analysis. Live mice were analyzed at Months 4, 6, and 8 body fat and lean weight using the EchoMRI machine. Carcasses at time of necropsy were analyzed for percent body fat, lean body mass, fat mass, and bone density using a GE Lunar Piximus II dual-energy X-ray absorptiometer (DXA). Body weights and caloric consumption was measured weekly in every mouse for the duration of the 90-week feeding regimen. A total of **360** MMTV-neu and wild-type (FVB) mice were used during Years 1 and 2 of this study.

In MMTV-neu non-ovariectomized (NOVX) mice, we found that the high fat diet regimen (HF) significantly increased body weight and percent body fat through increased caloric intake (HF vs. Control; $p < 0.0001$). The 30% caloric restriction (CR) significantly decreased body weight and percent body fat through decreased caloric intake (CR vs. Control; $p < 0.0001$) after 90 weeks of feeding. This finding was consistent and significant in age-matched wild-type FVB NOVX mice (**Figure 1**) ($n = 30/\text{group}$ for all groups). This feeding regimen was repeated in MMTV-neu ovariectomized (OVX) mice, and while Control-fed animals were typically heavier than their NOVX age-matched diet counterparts, HF animals were significantly heavier, and CR animals were significantly lighter, after 90 weeks of feeding. This was also consistent in wild-type FVB OVX animals ($p < 0.0001$ for all; $n = 30/\text{group}$) (**Figure 2**). Importantly, we found that after 90 weeks of feeding a high-fat diet-induced obesity regimen (60% kcal from fat), a control ad-libitum diet (10% kcal from fat), or a 30% calorie restriction regimen (reduction calculated versus Control caloric intake; isonutrient), CR significantly increased survival of MMTV-neu NOVX mice ($p = 0.01$). We found that HF diet did not further increase tumor risk in these animals versus Control caloric intake. Interestingly, we found that no tumors developed in MMTV-neu OVX mice, regardless of diet, after 90 weeks of feeding (**Figure 3**). After histopathological evaluation of the mammary fat pad whole mounts, we determined that this was likely due to a lack of mature branching in the mammary fat pad, thus a lack of tumor-initiating cells (data not shown). This can occur when ovariectomy is performed on young animals (4-6 weeks old), that do not have mature mammary glands, and the lack of estrogen prevents mammary gland maturation. In a subset of mice, which were sacrificed at 2 months ($n = 4/\text{group}$), we found that MMTV-neu mice begin to lose $\text{ER}\alpha$ expression very early in life. A significant difference in expression can be detected in mammary fat pad (MFP) between 6 week and 8 week-old mice ($p = 0.03$). In 2 month-old MMTV-neu mice, a trend exists linking CR to increased $\text{ER}\alpha$ expression and HF to decreased $\text{ER}\alpha$ expression. We also found that CR significantly increases $\text{ER}\beta$ expression. In fact, CR increases $\text{ER}\beta$ expression to levels even higher than those at the Baseline (6 week) timepoint, and was significantly increased from Control diet animals ($p < 0.0001$) (**Figure 4**). $\text{ER}\beta$ expression is thought to have a protective effect from breast cancer (6, 7), and could be a putative mechanism for CR-mediated cancer prevention in this model.

KEY RESEARCH ACCOMPLISHMENTS:

- High-fat, Control, and Calorie-Restriction diet regimens caused significant differences in body weights, caloric intake, and body composition of MMTV-neu OVX and NOVX mice.
- Calorie restriction significantly decreases tumor incidence in MMTV-neu NOVX mice.
- MMTV-neu NOVX mice began to lose $\text{ER}\alpha$ expression in mammary fat pad very early in life; a significant difference was detected between 6 week and 8 week-old mice.
- After 2 weeks of diet, CR significantly increased $\text{ER}\beta$ expression and a trend showed HF diet decreased $\text{ER}\beta$ expression in MMTV-neu NOVX mice.

REPORTABLE OUTCOMES: A manuscript resulting from this data is currently in preparation and will be submitted to Cancer Prevention Research.

SCIENTIFIC TRAINING: During the course of this fellowship (2009-present), I have attended 5 national meetings to present my work. These meetings include the American Association for Cancer Research Annual Meeting (3 meetings), the Aspen Cancer Conference, and the Era of Hope Conference. This month I have an invited platform presentation in Singapore at the International Epithelial-Mesenchymal Transition Meeting (October). In December, I also have an invited platform presentation at the San Antonio Breast Cancer Symposium. I have won numerous awards including the University of Texas Austin, Department of Toxicology 1-year Fellowship (NIH T32-ES007247), which I declined because I had already received the DOD fellowship. I have also won the University of Texas Austin, Department of Nutritional Sciences, Postdoctoral Fellow Research Award, the 2011 Betty Hay Travel Award to the 5th International Epithelial-Mesenchymal Transition Meeting in Singapore, and the 2011 American Association for Cancer Research Travel Award to the San Antonio Breast Cancer Symposium. I have also co-written 6 published manuscripts, and have 2 more that are currently under peer review. I am currently preparing 3 more manuscripts for publication that have not yet been submitted. I have been committed to my continuing education by attending the week-long National Cancer Institute Molecular Cancer Prevention Fellowship Course, held in Rockville Maryland (August 2011). My postdoctoral fellowship has contributed to my successes by providing funding to advance my research. This award had facilitated travel to many meetings and courses that I would not have been able to attend. I plan to continue my career in academic research by applying for a transitional research grant (through funding mechanisms like the NIH K99 or R00) in the next year. A grant will continue my history of funded research and allow me to successfully transition to an Assistant Professor position at a major university. My topic of research will continue to be elucidating the mechanism of energy balance effects on cancer.

CONCLUSION AND FUTURE RESEARCH DIRECTIONS: We have accomplished all tasks outlined in the original proposal for the first year of the study. We found that calorie restriction is a potent inhibitor of MMTV-neu tumor initiation, likely through decreased circulating IGF-I mediation of ER expression in the mammary fat pad. We are currently analyzing the full serum hormone panel of these mice, and elucidating the differences in signaling pathways downstream of the IGF-1 receptor and the estrogen receptor. Specific Aims 2 and 3 will be completed concurrently, and are currently in progress. We will use the MMTV-neu NOVX model to analyze the mechanism of obesity-driven tamoxifen resistance. Currently, no changes will be made to the study design of Specific Aims 2 and 3. However, we are considering using a new dual inhibitor of Phosphoinositide 3-kinase (PI3K) and AKT, which would inhibit the major downstream effectors of IGF-I, in lieu of the IGF-I inhibitor, which can often cause insulin resistance in animals. This inhibitor has not been tested clinically, and has very little pre-clinical work published on its effect on cancer. If we receive this drug, we will amend the protocol, and use the PI3K/AKT inhibitor instead of the IGF-I inhibitor for Aim 3.

This study will lay the foundation for larger translational/clinical studies investigating the role of IGF-I in promoting the adverse effects of obesity on breast tumor development and progression, and validating suppression of IGF-1 pharmacologically as an effective chemopreventive approach. A significant gap in the literature exists in elucidating the impact of circulating obesity-related hormones on the chemopreventive response to tamoxifen. The successful accomplishment of our proposed aims could have a significant impact on the development of prevention strategies for obesity-related breast cancer in high-risk women.

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3. A. C. Wolff *et al.*, *Arch Pathol Lab Med* **131**, 18 (2007).
4. S. D. Hursting, S. M. Smith, L. M. Lashinger, A. E. Harvey, S. N. Perkins, *Carcinogenesis* **31**, 83 (Jan, 2010).
5. J. D. Mosley, J. T. Poirier, D. D. Seachrist, M. D. Landis, R. A. Keri, *Molecular cancer therapeutics* **6**, 2188 (Aug, 2007).
6. A. Tsezou *et al.*, *Breast* **17**, 159 (Apr, 2008).
7. G. Lazennec, D. Bresson, A. Lucas, C. Chauveau, F. Vignon, *Endocrinology* **142**, 4120 (Sep, 2001).

APPENDICES: Curriculum Vitae for Sarah M. Dunlap, Ph.D.

Sarah M. Dunlap, Ph.D.

Formerly Sarah M. Smith

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Education

- | | |
|----------------------------------|--|
| <i>July 2008-present</i> | Postdoctoral Fellow , Department of Nutritional Sciences
University of Texas at Austin, TX,
Advisor: Dr. Stephen Hursting |
| <i>December 2007-May 2008</i> | Postdoctoral Fellow , Department of Pathology, University
of Texas M.D. Anderson Cancer Center, Houston, TX,
Advisor: Dr. Wei Zhang |
| <i>August 2003-December 2007</i> | Ph.D. , 2007, Cancer Biology, Graduate School of
Biomedical Sciences University of Texas Health Science
Center, Houston, Texas,
Advisor: Dr. Wei Zhang |
| <i>August 1999-May 2003</i> | B.S. , 2003, Genetics,
Texas A&M University, College Station, Texas |

Academic Awards and Honors

- 1999 Presidential Endowed Scholarship, Texas A&M University
- 1999 Brown Foundation Scholarship, Texas A&M University
- 1999 Dallas Morning-Star Scholar-Athlete Award
- 1999 Houston-Livestock Show and Rodeo Go-Texan Scholarship
- 1999 Robert C. Byrd Scholarship, State of Texas
- 1999 Lower Colorado Valley River Authority (LCRA) Scholarship
- 2003 Sigma Xi Honorable Mention poster presentation (Immunology Division), National Conference
- 2003 First Place poster presentation (Immunology Division), Student Research Week, Texas A&M University
- 2003 N.R. Bottino Outstanding Senior Student Research Award, Department of Biochemistry and Biophysics, Texas A&M University
- 2005-2007 Keck Center Pharmacoinformatics Training Program of the Gulf Coast Consortia (National Institutes of Health Grant 5 T90 DK070109-02) 3-year Fellowship, Houston, TX
- 2005 Unconditional pass on both written and oral portions of candidacy exam, Graduate School of Biomedical Sciences Cancer Biology Program, Houston, TX
- 2006 "Scholar-in-training" award for American Association of Cancer Research National Meeting, sponsored by Aflac Inc., Washington, DC

- 2006 GSBS Post-candidacy Travel Award for American Association of Cancer Research National Meeting, Washington, DC
- 2006 Third Place Platform Talk Cancer Biology Program Retreat, Graduate School of Biomedical Sciences Cancer Biology Program, Houston, TX
- 2006 Second Place abstract award winner (Basic Science Research Category, Graduate Division), Trainee Recognition Day, MD Anderson Cancer Center, Houston, TX
- 2006 American Legion Auxiliary Fellowship, Graduate School of Biomedical Sciences, Houston, TX
- 2007 Third Place Platform Talk Cancer Biology Program Retreat, Graduate School of Biomedical Sciences Cancer Biology Program, Houston, TX
- 2007 GSBS Post-candidacy Travel Award for American Association of Cancer Research National Meeting, Los Angeles, CA
- 2007 First place abstract award winner (Clinical/Translational Research Category, Graduate Division), Trainee Recognition Day, MD Anderson Cancer Center, Houston, TX
- 2007 NIH Travel award to the National Graduate Student Research Festival, Washington DC
- 2007 American Legion Auxiliary Fellowship, Graduate School of Biomedical Sciences, Houston, TX
- 2008 Trainee Excellence Award, MD Anderson Cancer Center, Houston, TX
- 2009 Department of Toxicology NIH Training Grant (T32-ES007247) 1-year Fellowship, University of Texas, Austin, TX
- 2009 Department of Defense (DOD) Breast Cancer Research Program (BCRP) of the Office of the Congressionally Directed Medical Research Programs (CDMRP) FY08 Postdoctoral Award (3-years, \$340,128), Department of the Army, Fort Detrick, MD
- 2010 Department of Nutritional Sciences, Postdoctoral Fellow Research Award, University of Texas, Austin, TX
- 2011 Betty Hay Travel Award to the 5th International Epithelial-Mesenchymal Transition Meeting in Singapore, awarded by The EMT International Association, Sydney, Australia
- 2011 American Association for Cancer Research Travel Award to the San Antonio Breast Cancer Symposium, San Antonio, TX

Publications

Dunlap SM and MacDonnell F. Chiral Ruthenium Polymer Synthesis. *Welch Summer Scholar Research Articles*. p23-28, 1998.

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*Co-first author.

**Publication resulted in cover image for this edition.

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Book Chapter

Hursting SD, **Smith SM**, Nogueira L, DeAngel R, Lashinger L, and SN Perkins. "Dietary Energy Balance, Calorie Restriction, and Cancer Prevention." Bioactive Food Components and Cancer. Pages 147-164. Ed. Milner JA and DF Romagin. Humana Press/Springer: New York, 2010.

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Research Experience

July 2008-Present: Postdoctoral Fellow, Department of Nutritional Sciences
University of Texas Austin (Dr. Stephen Hursting)

- Determine the effects of energy balance modulation on the breast cancer stem cell population
- Examine the effects of targeting the Akt/mTOR pathway to mimic calorie restriction in breast cancer prevention

December 2007-May 2008: Postdoctoral Fellow, Department of Pathology
University of Texas MD Anderson Cancer Center (Dr. Wei Zhang)

- Examined the role of integrin binding in IGFBP2-driven glioma progression
- Examined the role of Ink4a/Arf deletion in glioma progression
- Established stem cell assays in the laboratory for examination of the role of progenitor cells in the origin of glioma

August 2003-December 2007: Graduate Research Assistant, Department of Pathology
University of Texas MD Anderson Cancer Center (Dr. Wei Zhang)

- Designed Ph.D. project entitled, “Elucidation of the role of Insulin-like Growth Factor Binding Protein-2 (IGFBP-2) in glioma progression using a glial-specific transgenic mouse model.”

January 2000-May 2003: Undergraduate Research Assistant, Laboratory of
Biological Mass Spectrometry, Department of Chemistry, Texas A&M University
(Dr. David H. Russell)

- Analyzed the effect of detergents on peptide resolution using matrix-assisted laser desorption ionization (MALDI), using time-of-flight (TOF) as the main mass analyzer
- Pioneered a joint research project with Dr. Dickson Varner in the College of Veterinary Medicine that analyzed the semen protein content of sub-fertile stallions that have good sperm quality and motility versus the protein content of fertile stallions
- Analyzed the bacterial proteome of *E. coli* using HPLC and MALDI-TOF MS

May 2002-August 2002: Undergraduate Research Assistant, Laboratory for Antigen
Processing, Department of Immunology, Memorial Sloan-Kettering Cancer
Center, NYC (Dr. Lisa Denzin)

- Developed the protocol to generate H2-O α and H2-O β (mouse homologues of DO α and DO β) His-tagged fusion proteins

June 1998-July 1998: Welch Summer Scholar, Welch Summer Scholar Program,
University of Texas, Arlington (Dr. Frederick M. MacDonnell)

- Developed a protocol to synthesize a chiral ruthenium dimer, then connect the repeating unit to form a polymer
- Wrote a journal-style article entitled, *Chiral Ruthenium Polymer Synthesis*, which is published in a book with the other Welch research participants from around Texas

May 1997-August 1999: Laboratory Assistant, Department of Carcinogenesis,
Veterinary Division,
University of Texas MD Anderson Cancer Center (Dr. Tahir Rizvi)

- Aided in research focused on the regulation of viral gene expression and viral RNA packaging using human and simian immunodeficiency viruses (HIV/SIV) and Mason-Pfizer monkey virus (MPMV) as model systems.

Conference Presentations

2003 Sigma Xi National Conference, Poster presentation, Galveston, TX “The isolation and purification of H2-O α and H2-O β (mouse homologues of DO α and DO β) His-tagged fusion proteins”

2003 Student Research Week, Poster presentation, Texas A&M University, College Station, TX. “The isolation and purification of H2-O α and H2-O β (mouse homologues of DO α and DO β) His-tagged fusion proteins”

2005 American Association of Cancer Research Annual Meeting, Poster presentation, Anaheim, CA. “The putative tumor suppressor Shrew-1 gene on chromosome 1p36 inhibits adhesion and migration of glioma cells”

- 2005 M.D. Anderson Trainee Recognition Day, Poster presentation, Houston, TX. “The putative tumor suppressor Shrew-1 gene on chromosome 1p36 inhibits adhesion and migration of glioma cells”
- 2005 2nd Annual Conference for Tumor Progression and Therapeutic Resistance, Platform presentation, Boston MA. “IGFBP2 causes glioma progression *in vivo*”
- 2005 15th Annual Keck Center Research Conference, Poster presentation, Houston, TX. “IGFBP2 causes glioma progression *in vivo*”
- 2005 Graduate School of Biomedical Sciences Annual Poster Competition, Poster presentation, Houston, TX. “IGFBP2 Actively Contributes to Glioma Initiation and Progression in the RCAS-tva Glial-specific Transgenic Mouse Model System”
- 2006 United States and Canadian Society of Pathology Annual Meeting, **Platform presentation**, Atlanta, GA. “IGFBP2-Associated Diffuse Glioma Initiation and Progression Demonstrated in the RCAS-tva Mouse Model System”
- 2006 Graduate School of Biomedical Sciences Cancer Biology Program Retreat, **Platform presentation**, Houston, TX. “IGFBP2-Associated Diffuse Glioma Initiation and Progression Demonstrated in the RCAS-tva Mouse Model System”
- 2006 American Association of Cancer Research Annual Meeting, **Platform presentation**, Washington D.C. “Tissue-specific transgenic mouse model experiments demonstrate that IGFBP2 actively contributes to glioma development and progression”
- 2006 M.D. Anderson Genomics Mini-Symposium, **Platform presentation**, Houston, TX. “IGFBP2: From genomic marker to functional characterization.”
- 2006 M.D. Anderson Trainee Recognition Day, Poster presentation, Houston, TX. “Tissue-specific transgenic mouse model experiments demonstrate that IGFBP2 actively contributes to glioma development and progression.”
- 2006 16th Annual Keck Center Research Conference, Poster presentation, Houston, TX. “Tissue-specific transgenic mouse model experiments demonstrate that IGFBP2 actively contributes to glioma development and progression.”
- 2006 AACR Special Conference on Mouse Models of Cancer, Poster presentation, Cambridge, MA. “Tissue-specific transgenic mouse model experiments demonstrate that IGFBP2 actively contributes to glioma development and progression.”
- 2006 Society for Neuro-Oncology 11th Annual Meeting, **Platform presentation**, Orlando, FL. “The *SHREW1* gene, frequently deleted in oligodendrogliomas, functions to inhibit cell adhesion and migration.”
- 2006 Society for Neuro-Oncology 11th Annual Meeting, **Platform presentation**, Orlando, FL. “Tissue-specific transgenic mouse model experiments demonstrate that IGFBP2 actively contributes to glioma development and progression.”
- 2007 Graduate School of Biomedical Sciences Cancer Biology Program Retreat, **Platform presentation**, Houston, TX. “Iip45 attenuates IGFBP2-driven glioma progression and sensitizes glioma cells to DNA damage-induced cell cycle arrest and apoptosis.”
- 2007 Graduate School of Biomedical Sciences Annual Poster Contest, Poster presentation, Houston, TX. “IGFBP2 activates the Akt pathway and collaborates with K-Ras or PDGFB in gliomagenesis and glioma progression”

- 2007 American Association of Cancer Research Annual Meeting, Poster presentation, Los Angeles, CA. "Iip45 attenuates IGFBP2-driven glioma progression and sensitizes glioma cells to DNA damage-induced cell cycle arrest and apoptosis"
- 2007 M.D. Anderson Trainee Recognition Day, **Platform presentation**, Houston, TX. "IGFBP2 activates the Akt pathway and collaborates with K-Ras or PDGFB in gliomagenesis and glioma progression"
- 2007 The National Institutes of Health (NIH) National Graduate Student Research Festival, Poster presentation, Washington D.C. "Insulin-like Growth Factor Binding Protein 2 promotes glioma development and progression via Akt pathway activation"
- 2007 The University of Texas Health Science Center Houston Annual Research Day, Poster presentation, Houston, TX. "Insulin-like Growth Factor Binding Protein 2 promotes glioma development and progression via Akt pathway activation"
- 2008 American Association of Cancer Research Annual Meeting, Poster presentation, San Diego, CA. "Integrin binding is essential for IGFBP2-driven glioma progression"
- 2009 American Association of Cancer Research Annual Meeting, Poster presentation, Denver, CO. "Wnt-1 mammary tumors are enriched in CD44⁺/CD24⁻ cells with cancer stem cell characteristics"
- 2009 Aspen Cancer Conference, Invited Trainee/Poster presentation, Aspen, CO.
- 2009 American Association of Cancer Research Frontiers in Cancer Prevention Research Meeting, Houston, TX. "Aggressiveness of MMTV-Wnt-1 mammary tumors corresponds with enrichment of a CD44⁺/CD24⁻ putative cancer stem cell population"
- 2010 American Association of Cancer Research Annual Meeting, Poster presentation, Washington, D.C. "Enrichment of a CD44⁺/CD24⁻ putative cancer stem cell population drives tumor progression and growth factor independence in a mouse model of post-menopausal breast cancer."
- 2011 American Association of Cancer Research Annual Meeting, Poster presentation, Orlando, FL. "Obesity drives EMT and tumor progression in a novel Wnt-1 mammary cancer model."
- 2011 Era of Hope Conference- Military Health Research Forum, Poster presentation, Orlando FL. "Calorie restriction significantly prevents spontaneous tumor formation in the MMTV-neu mouse model of human luminal-A type breast cancer."
- 2011 5th International Epithelial-Mesenchymal Transition Meeting, **Platform presentation**, Biopolis, Singapore. "Obesity Promotes Epithelial-To-Mesenchymal Transition and Tumor Progression in a Syngeneic Mouse Model of Claudin-Low Breast Cancer."
- 2011 San Antonio Breast Cancer Symposium, **Platform presentation**, San Antonio, TX. "Obesity drives epithelial-to-mesenchymal transition and tumor progression in a novel claudin-low mammary cancer model."

SUPPORTING DATA:

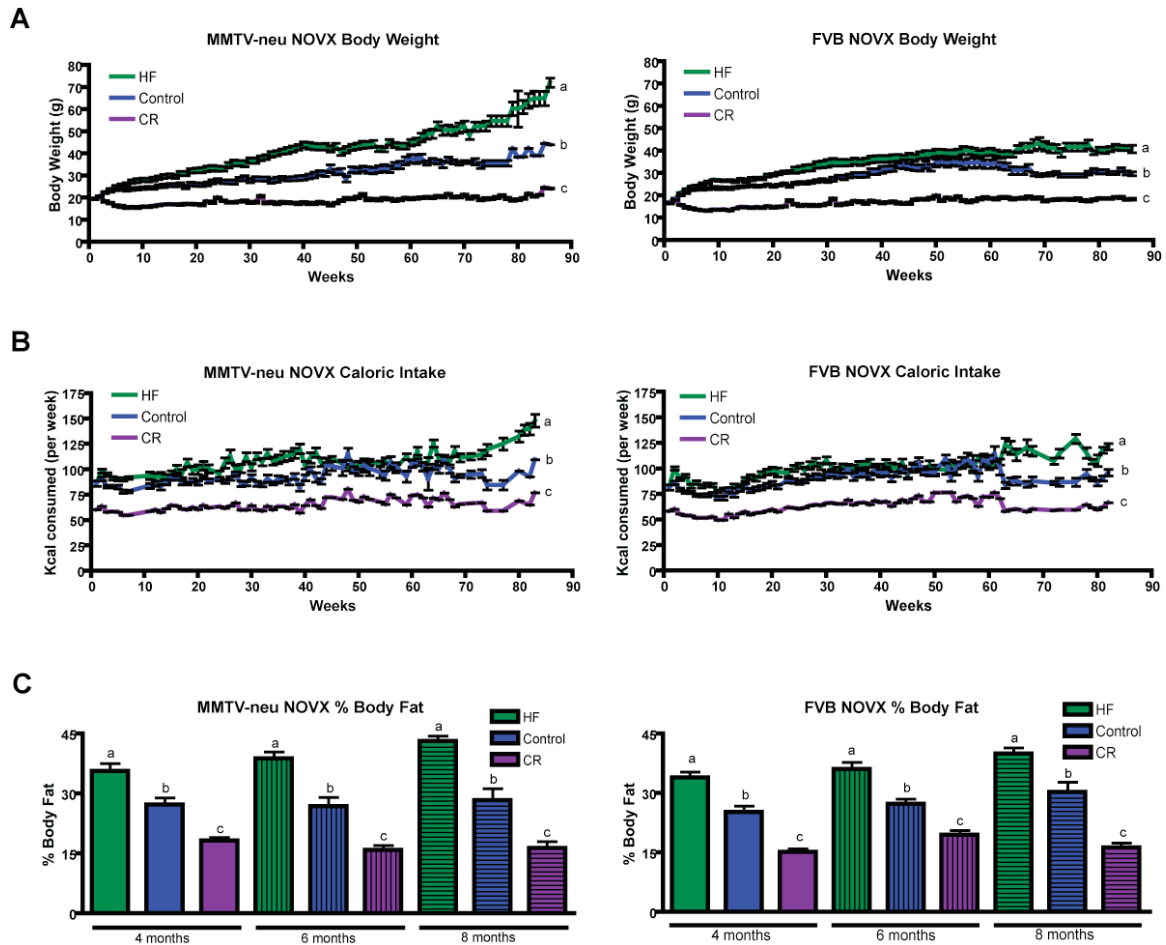


Figure 1. Body Weights, Caloric Intake, and Body Composition of non-ovariectomized (NOVX) mice. (A) Body weights for MMTV-neu NOVX and age-matched non-transgenic (FVB NOVX) controls. Body weights were recorded weekly (AVG $\pm$ SEM). CR mice have the lowest body weights and those consuming the HF diet, the highest body weights. (B) Caloric intake graphs: caloric intake was recorded weekly, (AVG $\pm$ SEM). The CR mice consumed the least amount of calories, while the CON and HF mice consumed roughly 30% more per week. (C) % Body fat graphs; body compositions were analyzed at 4, 6, and 8 months (AVG $\pm$ SEM). HF mice in all groups show highest percentage body fat, while the CR mice show the lowest percentage body fat. Different letters represent significant differences between groups ($p < 0.0001$). All mice received a sham surgery at 5-6 weeks of age ($n = 30/\text{group}$).

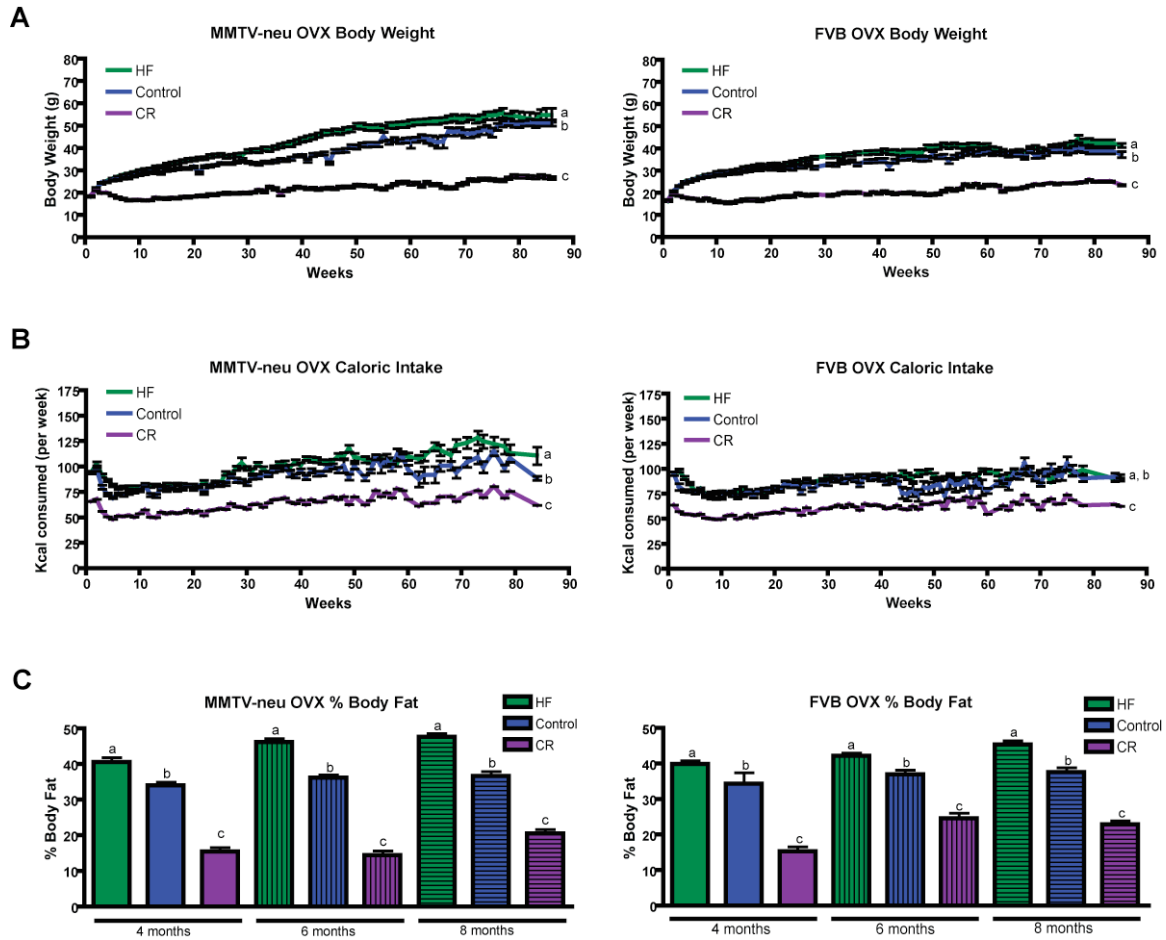


Figure 2. Body Weights, Caloric Intake, and Body Composition of ovariectomized (OVX) mice. (A) Body weights for MMTV-neu OVX and age-matched non-transgenic (FVB OVX) controls. Body weights were recorded weekly (AVG $\pm$ SEM). CR mice have the lowest body weights and those consuming the HF diet, the highest body weights. (B) Caloric intake graphs: caloric intake was recorded weekly, (AVG $\pm$ SEM). The CR mice consumed the least amount of calories, while the CON and HF mice consumed roughly 30% more per week. (C) % Body fat graphs; body compositions were analyzed at 4, 6, and 8 months (AVG $\pm$ SEM). HF mice in all groups show highest percentage body fat, while the CR mice show the lowest percentage body fat. Different letters represent significant differences between groups ($p < 0.0001$). All mice received an ovariectomy surgery at 5-6 weeks of age ($n = 30/\text{group}$).

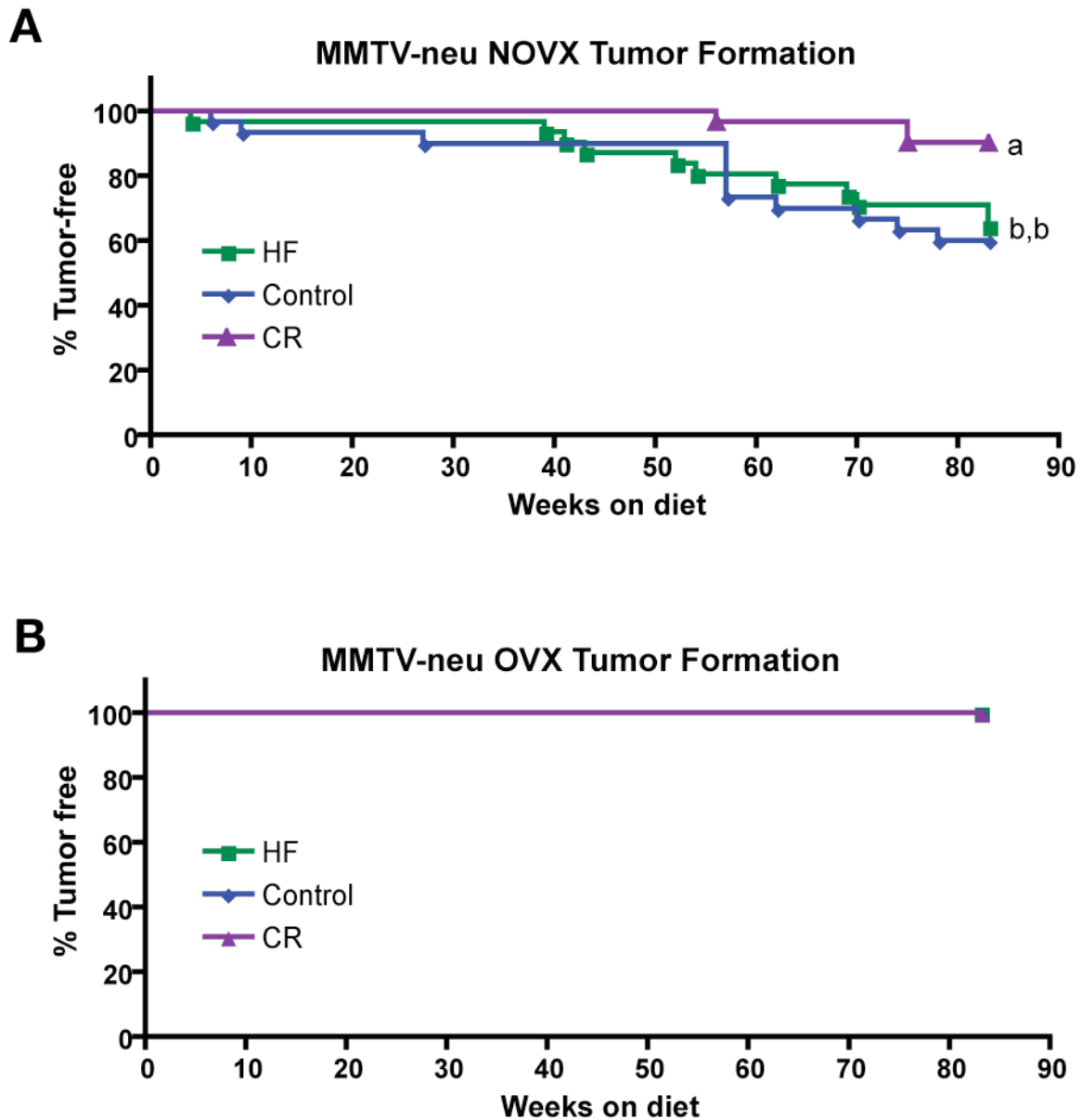


Figure 3. Calorie restriction significantly decreases tumor incidence in MMTV-neu NOVX animals. (A) MMTV-neu NOVX mice were placed on 3 diets (n=30/group) for 60 weeks: high fat diet, which induces obesity (HF; 60% kcal from fat), control diet which induces a slightly overweight animal (10% kcal from fat), and 30% calorie restriction (CR; 30% reduction in calories from Control ad-libitum diet). CR significantly decreased tumor incidence after 60 weeks of feeding ($p=0.01$). (B) MMTV-neu OVX mice were placed on the same 3 diets (n=30/group) for 60 weeks, and diet was found to have no effect on tumor incidence.

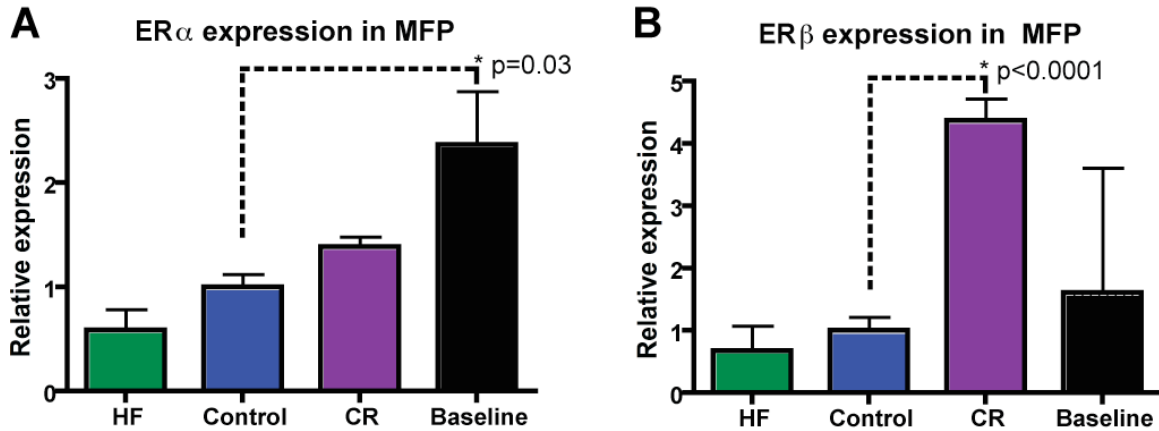


Figure 4. ER α / β mRNA expression in MFP of 8 week-old MMTV-neu NOVX mice.

(A) ER α relative expression in MMTV-neu mice. Baseline animals were 6 weeks/old (chow diet); diet group mice were 8 weeks/old. There was a significant loss of ER α expression in the older diet group mice (Baseline vs. Control; p=0.03). (B) ER β relative expression at same ages as Panel A. CR significantly increases ER β expression (CR vs. Control; p<0.0001). Data is AVG $\pm$ SEM, n=5/group

Statistical Analysis: One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant differences test was used to assess the effects of diet on body weight, caloric intake, and body composition. Unpaired student's t-test was used to assess differences in gene expression, and the Kaplan-Meier test was used to assess differences in survival curves.