POSTER SESSION WITH DESSERT
NOVEMBER 8
7:30-9:00 PM
Presidential Ballroom III - Third Level

In the Poster Session, researchers and advocates present their research findings, program summaries and works in-progress. View the posters and talk with the authors about their work.

Posters can be mounted anytime after 12 noon on November 8 and taken down by 1:00 pm November 9.

Study Helpers: Breast Cancer Advocates Assist with the Growing Up Female Study of Young Girls

Wendy Anderson[1], Banita Bailey[2], Kathleen Ball[1], M. Kathryn Brown[3], Ann Hernick[1], Andrea Ice[3], Peggy Monroe[2,4]. [1]Breast Cancer Alliance of Greater Cincinnati; [2]Pink Ribbon Girls; [3]University of Cincinnati College of Medicine; [4]Helping Each Other Support Group.

One of the objectives of the Cincinnati Community Outreach and Translation Core has been to provide interested breast cancer advocates opportunities to assist with the Cincinnati Breast Cancer and the Environment Research Center study of young girls called Growing Up Female. The Study Helper program was developed to afford breast cancer advocates opportunities to learn more about the Growing Up Female study protocol and to assist with defined study activities. The Study Helper program was established in 2004 by the COTC in conjunction with our Advocate Advisor, the Growing Up Female study staff and the Department of Volunteer Services at Cincinnati Children's Hospital Medical Center (CCHMC).

Study Helpers have been recruited from local breast cancer advocacy, education and support organizations and by word of mouth. The application to become a CCHMC volunteer includes an extensive application process, medical screening, interview and orientation. The orientation conducted by the Director of Volunteer Services is coupled with an overview of the Growing Up Female study protocols by project investigators. Semi-annual study updates are provided to the Study Helpers and other Cincinnati BCERC volunteers. Study Helpers come with a diversity of backgrounds: many are survivors, most are mothers, and some are health professionals, but everyone has a personal interest in breast cancer research.

Study Helpers perform a number of study-related activities. The Study Helpers marvel at the courage the girls demonstrate in their participation in the study; and the girls relish the attention and comfort provided by the Study Helpers. The tasks performed by the Study Helpers improve the efficiency of the study sessions, allowing the study staff to focus on data collection procedures. Study Helpers act as *extra sets of eyes and ears*, whether at the study sessions, during data review or interpreting results. Processing changes recommended by Study Helpers have improved interactions between study participants and staff and cut down on errors. Parents attending study sessions with their daughters look to the Study Helpers for information about study logistics.

This poster will present data pertaining to the number of volunteers completing the application-orientation-training process; the number of study sessions attended by Study Helpers; and reasons for withdrawing from the program. The activities performed by the Study Helpers will be described. Plans to evaluate Study Helpers' and the Growing Up Female study staff's satisfaction, knowledge gained and suggestions for improvements will be presented.

Hormonal Regulation and Function of Progesterone Receptor Isoforms in the Pubertal Mouse Mammary Gland

Mark D. Aupperlee and Sandra Z. Haslam, Ph.D.
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The role of progesterone (P) in mammary gland proliferation leading to alveologenesis during pregnancy has been well established. However, less is known about a potential role of P during pubertal mammary gland development. Importantly, a role for P in breast cancer has been implicated in studies of the mouse and human breast. Given that puberty is an important period of breast cancer susceptibility, it is critical to elucidate the role and function of P in the mammary gland during puberty. Thus, in the present study we investigated hormonal regulation of progesterone receptor isoforms A (PRA) and B (PRB) expression and their functional roles in proliferation in pubertal Balb/c mice. Pubertal (6-week-old) ovariectomized mice were treated for 5 days with vehicle control (C), estrogen (E), P, or E+P. The formation of end buds and an increase in proliferation were observed in E, P, and E+P treated mice. Most notably, P alone was able to stimulate proliferation in end buds, the major growth point of the gland. Additionally, P-induced sidebranching, the prominent response in adult mice, was almost absent in pubertal mice. In end buds E induced proliferation of primarily PRA negative cells, whereas P induced significantly more proliferation of PRA positive cells. In summary, we have made the novel observation that P has a positive proliferative effect in the end buds of the pubertal mammary gland and does not induce sidebranching. These effects of P in the pubertal gland are distinct from its role in the adult. It has been noted that a portion of label retaining cells, a marker of potential stem/progenitor cells, are PR positive. The results also lead us to speculate that P may be involved in the proliferation of PRA positive mammary stem/progenitor cells in the pubertal gland.

Relationships Among Parental Depression, Family Dysfunction, Child Psychopathology, BMI and Tanner Staging: an Interim Analysis of Second Year Data

Erin Baker, M.S. Kim Dietrich, Ph.D., M.A., Frank Biro M.D. The University of Cincinnati College of Medicine and Academic Health Center, Department of Environmental Health, Division of Epidemiology and Biostatistics. Cincinnati Children's Hospital Medical Center, Division of Adolescent Medicine.

Background: Participants and families of Growing Up Female were asked to respond to questionnaires which describe parental depression, family dysfunction, as well as child psychopathology. The results of these questionnaires were compared to data on the participant's growth and maturation. Interim cross-sectional data from second year visits (2B) to the Cincinnati BCERC were analyzed for associations between parental depression, family dysfunction, child psychopathology and the participant's measures of BMI and maturation staging of secondary sexual characteristics. **Methods:** The Cincinnati site of the BCERC examines participants twice a year, administering questionnaires at the time of the second visit of each study year. The Center for Epidemiologic Studies Depression Scale (CESD) is filled out by a parent/guardian. The Family Environment Scale, also filled out by the parent, describes the family environment, including measures of conflict, control, cohesion, and expressiveness. The Behavior Assessment System for Children (BASC) is filled out by a parent, and provides indices of internalizing and externalizing behaviors as well as adaptive skills and a global index of behavioral symptoms. The Children's Depression Inventory (CDI) is filled out by the girl. **Results**: At the time of the analysis of second year data, ten referrals had been made based on CESD scores (n=158); ten referrals had been made based on BASC scores (n=160); and five referrals had been made based on CDI scores (n=178). There were 136 participants who had completed all four questionnaires at the time of analysis. We found internalizing psychological symptoms of the participant to be strongly associated with body mass index, maternal depression; and higher scores in conflict and control in the family. Maturation staging was not found to be associated with internalizing or externalizing psychological symptoms nor depression in the child. We found that higher scores on the self-rating measure of child psychopathology were associated with African American race. **Conclusion**: Future work will incorporate longitudinal data analyses to better understand the relationships of child and maternal psychopathology, family dynamics and the child's path through puberty.

Advocate Research Training (ART)

Kathleen M. Ball, RN - Breast Cancer Alliance of Greater Cincinnati; Ann Hernick - Breast Cancer Alliance of Greater Cincinnati; Deborah Clegg, PhD - University of Cincinnati College of Medicine; M. Kathryn Brown, PhD - University of Cincinnati College of Medicine

Background

Historically, breast cancer advocates have not participated in setting and/or conducting breast cancer research agendas. There is growing demand for breast cancer advocates who are trained, educated and represent a patient constituency to be meaningfully involved in all aspects of decision-making that affect breast cancer research. The Department of Defense (DOD) Peer Review Breast Cancer Research Program has involved breast cancer advocates in the review of research proposals and the presentation of findings since its inception in 1992. Along with a right to meaningful participation comes a responsibility to be educated. While there are courses offered nationally, such as the National Breast Cancer Coalition Fund's (NBCCF) Project LEAD®, that teach the science of breast cancer, we have found barriers exist that prohibit advocates from participation. Obstacles such as cost, time away from work and family, and a feeling of intimidation hinder many advocates from committing to such training.

Work Performed

The Breast Cancer Alliance of Greater Cincinnati (BCA), working with the Cincinnati Breast Cancer and the Environment Research Center (BCERC), has developed a program to educate and train local breast cancer advocates in the fundamentals of breast cancer research. The program is called Advocate Research Training (ART). Participants are recruited through their involvement with the BCA and the Cincinnati BCERC. The goal of ART is to educate more breast cancer advocates in both human and animal research study methods so that they can confidently participate in programs such as the NBCCF Project LEAD® and the DOD Peer Review Breast Cancer Research Program. This participation will also help them to be more prepared to contribute to the biology and epidemiology studies within the Cincinnati BCERC.

The ART program includes educational methods and training exercises that are geared to persons with little science background and affords participants opportunities to ask questions in comfortable settings. Relevant research papers are circulated with a glossary for definitions. A half-day science course focuses on epidemiology and biology research methods, hands-on lab exercises, and behind-the scenes tours of the animal facilities.The educational/training program will be described in detail.

Results and Next Steps

Evaluations from the pilot project and the first ART session will be summarized. A CD created with videotapes of the scientific presentations that is being made available to other interested advocates will be presented. We will also describe a collaborative year-long study project, beginning with the generation of a hypothesis thru publication of findings.

Prenatal Bisphenol A Exposure Alters the Mammary Proteome in Prepubertal Rats

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Background: Bisphenol A (BPA) is a high-production-volume chemical used in the manufacture of polycarbonate plastics and epoxy resins. It is present in many commonly used products including food and beverage containers, baby bottles, and dental composites. The detection of BPA in 95% of human urine samples clearly attests to the widespread use of BPA and widespread human exposure to BPA (Calafat *et al.*, 2005). Although the effects of BPA on reproductive function have been studied extensively, there is still controversy regarding the effects of its effects in the development of the mammary gland and possible involvement in cancer causation later in life.
Objectives: In this study, a proteome analysis using 2D gels and mass spectrometry (MALDI-TOF- TOF) was performed to identify proteins differentially regulated by prenatal BPA exposure. **Methods**: Pregnant Sprague-Dawley CD rats were fed phytoestrogen-free AIN-76A diet and gavaged from post-conception day 10 until birth with 1) sesame oil (control), 2) 25 µg/kg BW BPA (low BPA), or 3) 250 µg/kg BW BPA (high BPA). Mammary gland was collected at 21 days postpartum.
Results: Twenty proteins were identified as either up-regulated or down-regulated. They included proteins related to cancer, cytoskeleton components, proteins involved in transcription, oxidative stress, metabolism, immune function, DNA repair, RNA processing, and vesicle transport. Among the cancer related proteins, CDK5 regulatory subunit-associated protein 3, Rab GDP dissociation inhibitor beta (Rab GDI-beta), and vimentin were down-regulated. CDK5 regulatory subunit-associated protein 3 modulates Cdk1/cyclin B1 function, which in turn regulates the transition through late G2 and mitosis. Rab GDI-beta regulates the GDP-GTP exchange reaction of members of the rab family, small GTP-binding proteins of the ras super family. Reduced expression of Rho-GDIs has been observed in breast tumor tissues and it has been suggested that unbalanced Rho-GDIs in breast cancer could have clinical significance. Of particular interest is that prenatal BPA exposure induced up-regulation of gamma-synuclein (breast cancer-specific protein 1). Gamma synuclein is dramatically up-regulated in the vast majority of late-stage breast and ovarian cancers and over-expression of this protein has been shown to enhance tumorogenicity.
Conclusions: Although further analysis and validation of the proteins identified by MALDI-TOF- TOF is necessary, this study highlights the value of this methodology in advancing in the understanding of the possible molecular events that could be affected by BPA during mammary gland development.
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Pubertal Assessment Methodology and Baseline Characteristics in the BCERC Cohort

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Background: Puberty may serve as a sensitive indicator to the impact of environmental exposures. Determinants of puberty which may be risk factors for breast cancer and other chronic diseases are not entirely established. Genetics and environment are thought to play roles in pubertal onset. Methods of pubertal assessment require attention to breast morphology as well as standardization for multiple assessors.
Objectives: This paper describes the methods and training of research personnel for determining pubertal stages as well as the characteristics and maturation status of the cohort of girls at baseline, and maturation status of girls at age 7 years.
Methods: Methodology for pubertal maturation staging, based on the Tanner puberty stages, was developed collaboratively among investigators from all three BCERC sites. Girls were recruited through three centers: Mount Sinai School of Medicine (schools and neighborhood health centers), Cincinnati Children's Hospital (schools and breast cancer registry), and Kaiser-Permanente (HMO membership). Baseline characteristics were obtained by interview and by anthropometric measurements. For these analyses, development of breast tissue was used as the sign of pubertal maturation.
Results: The baseline cohort included over 1200 girls. There were significant differences in pubertal status by race and ethnicity between the three sites, as well as baseline body mass index (BMI). At age 7, the proportion of girls who had attained breast stage 2 or greater was 122/919, or 13.2%. The proportion of girls who had breast maturation at age 7 varied by site, BMI, and race/ethnicity.
Conclusions: The proportion of girls recruited in the BCERC who had breast development at age 7, 13.2%, is greater than that reported from studies of girls born 20-30 years earlier. For example, the PROS study published by Herman-Giddens in 1997, utilizing similar maturation assessment techniques, reported that at age 7.5% of white and 15.4% of black participants had attained breast development. Future analyses of the BCERC cohort, using longitudinal approaches and the wealth of anthropometric, lifestyle, psychological, family history, genetic, and chemical exposure data that is being collected, may help to define further the factors associated with onset of puberty.

Effect of irradiation over the proliferating cell population of the mouse mammary gland

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Our principal goal is to study how ionizing radiation (IR), which is a known breast carcinogen, affects the stem cell population in the mouse mammary gland. The mouse mammary gland epithelium consists of a layer of cells in contact with the lumen and a basal layer of myoepithelial cells with contractile capacity. These two cell types have a common progenitor or putative adult stem cell (ASC). ASC intermittently cycle, which is a characteristic thought to contribute to the accumulation of damage and neoplasic transformation.

It is known that p53 knockout mice present a higher frequency of cancer. Previous data obtained in our lab by clearing fat-pad and serial transplantation showed increased repopulation ability for p53 het when compared with WT tissue. Our hypothesis is that the altered p53 genotype could have an increased frequency of cancer because of an increased presence of stem cell. Moreover, p53 null mammary gland fragments transplanted into cleared fat-pads of WT and TGF-□(+/-)IR host mice presented an increased frequency of cancer. This suggests that IR alters the microenvironment by raising the tumor frequency. Our hypothesis is that IR could alter the microenvironment, causing the regulation of the stem cells to be affected, increasing their numbers and the chances to generate a cancer.

Our main objectives are two: to determine if the p53 null and p53 het mice present a higher frequency of stem cells than the WT mice; to study the effect of the IR over the stroma, which could alter the stem cell control by increasing the number of stem cells and apparition of cancer.

Flow cytometry is used to determine the presence of putative stem cell markers $CD24^{+}$ $CD49f^{hi}$ on WT, p53 het and p53null epithelial cells extracted from 8 week old mice. The stem cell populations will be compared to show possible differences among the genotypes.

A potential higher presence of stem cells in p53 knockout mice will be tested using a limiting dilution assay. Single epithelial cells will be transplanted in limiting dilution concentrations into cleared fat-pads of 3 week old Balb-c mice. A higher frequency of stem cells will be reflected with an increased repopulation capacity of the corresponding genotype.

Finally, WT, p53het and p53null tissue will be transplanted into sham and IR host. These mice will be labeled with bromodeoxyuridine (BrdU) using a classic pulse-chase labeling protocol to identify labeling retaining cells (LRC). The LRC population, which contains a subpopulation of slow cell cycling stem cells, will be analyzed and compared to determine the effect of IR.

Preliminary data will be presented and discussed.

Prepubertal Exposure to DDT Stimulates the Earlier Onset of *neu*-induced Mammary Tumors in Mice

Vicki L. Davis[1,2], Nakpangi A. Johnson[1], Claude L. Hughes[3], and Warren G. Foster[4]. [1]Graduate School of Pharmaceutical Sciences, Duquesne U.; [2]Ctr. for Environmental Oncology, U. of Pittsburgh; [3]Consortium for Molecular Epidemiology, Genomics, Environment and Health, Research Triangle Institute, RTP, NC; and [4]Dept. of Obstetrics & Gynecology, McMaster University.

Background: The estrogenic activity of some DDT compounds raises concern for breast cancer risk since DDT is persistent, bioaccumulates, and is stored in the fat that envelopes the hormonally-responsive breast tissue. Although most concern centers on the weak estrogenic actions of some DDT compounds, other metabolites possess antiandrogenic activity, including the most prevalent congener, p,p' DDE. However, no studies have investigated whether the local stores of these endocrine disruptors influence cancer development in the neighboring mammary tissue. Epidemiological studies on older women provide mixed results regarding whether DDT influences breast cancer risk; however, if the window of exposure is important for its effects, the ability to examine consequential effects of early DDT exposure on breast carcinogenesis is essentially unattainable.

Hypothesis/Objectives: The goal of this study was to determine if an antiandrogenic (p,p' DDE) and/or an estrogenic (o,p' DDE) DDT metabolite stored locally in the immature mammary gland influences tumor development in adult mice that mimic $HER2^{+}$ breast cancer. We hypothesized that p,p' DDE would increase mammary tumorigenesis more than its estrogenic isomer due to its prevalence and stronger hormonal (antiandrogenic) activity.

Methods/Work Performed: To examine the effects of locally stored DDT compounds, slow-release pellets containing p,p' DDE or o,p' DDE (5 μg/pellet) were surgically implanted in the mammary fat pads of 3-week-old MMTV-*neu* mice maintained on an isoflavone-free diet. Tumor outcomes were assessed in aging females until 14 months of age.

Results: Mammary tumor latency was significantly shorter in p,p' DDE-treated females, but not in those exposed to o,p' DDE. The levels of *neu* transgene expression in mammary tissue were not significantly modified by either treatment, suggesting that the p,p' DDE-induced early onset of tumors is not due to artificial stimulation of *neu* transgene expression through effects on the MMTV promoter.

Conclusions: These results demonstrate that local accumulation of the antiandrogenic p,p' DDE accelerates mammary tumor development induced by the most common oncogene in breast cancer (HER2/*neu*). The timing of exposure early in mammary gland development may be critical for this effect as p,p' DDE levels diminish with age into the lowest range detected in mature women (<30μg/kg lipid). Therefore, our results may furnish a possible explanation for the lack of conclusive evidence linking DDT and breast cancer risk since it is unknown what levels of exposure occurred at sensitive windows in breast development of the older women.

C/EBPβ Regulation of Progesterone Receptor Expression and Progesterone Regulation of C/EBP Expression: A Potential Regulatory Axis in Mammary Gland Development

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C/EBPβ is a critical transcription factor in the regulation of mammary gland proliferation and development. Experiments with C/EBPβ knockout mice have demonstrated a requirement of C/EBPβ for ductal morphogenesis and alveologenesis. C/EBPβ has been observed to occur in three isoforms in mammary and other tissues: C/EBPβ p39 (LAP-1) and C/EBPβ p36 (LAP-2), both potent transcriptional activators, and C/EBPβ p20 (LIP), a truncated form lacking classical transactivation domains and generally reported to be an inhibitor of C/EBP-dependent transcription. Studies of C/EBPβ isoform expression in rat and murine mammary development have reported that the hallmark of increased C/EBPβ expression during pregnancy is a 100-fold increase in LIP expression. Progesterone is a critical factor for proliferation and alveologenesis in the adult mammary gland, and experiments with progesterone receptor B (PRB) knockout mice show a requirement of PRB for alveologenesis. We hypothesized that progestin signaling through PRB and C/EBPβ expression act in the same pathway to regulate mammary gland development. We performed Western analyses of C/EBPβ isoform expression in the pregnant murine mammary gland, progestin-treated mammary gland of ovariectomized mice, progestin-treated mammary tumor cell lines and found induction of LIP expression in all. Transient cotransfection into a mammary tumor cell line of progesterone receptor promoter-reporter constructs with expression vectors that individually express C/EBPβ isoforms revealed that all isoforms of C/EBPβ, surprisingly including LIP, could transactivate the progesterone receptor promoter. Furthermore, immunofluorescence studies suggest that C/EBPβ and progesterone receptor A (PRA) expression are mutually exclusive in pregnant mammary epithelium. The increase in LIP expression with either pregnancy or progestin treatment and the mutually exclusive expression of C/EBPβ and PRA lead us to hypothesize that LIP expression directly promotes the expression of progesterone receptor B (PRB).

Characterization of RNA transcripts from the mouse progesterone receptor gene

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The 5'-ends of the human and rat PR transcripts were previously mapped by Kastner *et al.,* 1990 and Kraus *et al.* 1993, respectively, but they have yet to be delineated for the mouse PR gene. Additionally, the existence of one or two promoters needs to be confirmed for mouse PR, since this is central to the question of whether or not there is differential regulation of the PRA and PRB isoforms occurring at the transcriptional level. Experiments using primer extension to identify start sites for PR transcription within uterine RNA suggest that the PRA promoter may, in fact, lie downstream of the region we've previously analyzed by RNase protection, dispersed over a 440 bp region beginning approximately 150 bp upstream of the PRB ATG initiation codon, with the majority of sites actually occurring where they would be expected, downstream of the PRB ATG. Similarly, we have been unable to observe PRB transcripts corresponding to the predicted +1 start site, but instead see evidence of transcription initiation being dispersed over a broad region from +114 to +372 bp located 260 bp upstream of the PRB ATG. If confirmed, this would represent an additional difference comparing mouse PR with the human and rat systems, and increase the likelihood that, at least in the mouse, a shift in transcription from the proximal PRA promoter to the distal PRB promoter may account for the loss of PRA expression and the appearance of PRB that accompanies lobulo-alveolar development during pregnancy. Efforts to confirm these results using RNA extracted from mouse uterus and mouse mammary gland will continue, and will be extended to include several mouse mammary tumor cell lines that are reported to be both steroid receptor positive and estrogen or progestin responsive. If primer extension is not sufficiently sensitive to measure PRA and PRB mRNA in mouse mammary tissue and cell lines, then quantitative PCR will be used. Additionally, Northern blotting will be used to provide quantitative information on the relative levels of total PR mRNA and how they change following hormone treatment.

"Let's Ask the Children": Using semi-structured face to face interviews to assess minor aged daughters' experiences with participation in a breast health feasibility study.

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Background: Mounting evidence suggests genetic factors and environmental exposures in childhood/ adolescence may affect breast cancer development in adulthood. The Ontario Familial Breast Cancer Registry (OFBCR) is one of 6 international sites collaborating in the Breast Cancer Family Registry (Breast CFR), established in 1995 by the National Cancer Institute to provide a collaborative infrastructure for interdisciplinary studies of the genetic and environmental epidemiology of breast cancer. To facilitate the identification and characterization of early-life risk factors for breast cancer, the Breast CFR plans to expand its adult cohort to include female youth ages 5-17 years. To assess the feasibility of youth cohort expansion, we will invite a cross-section of 300 daughters ages 7-17 years of parents already enrolled in the OFBCR to participate in a pilot study that will measure breast development using optical spectroscopy (OS), and breast Tanner Staging (TS). Participants will be invited to complete a mailed questionnaire and attend a 1 hour clinic visit involving an exercise interview, anthropometric measurements, blood sampling, breast exam for pubertal staging (TS) and OS measures.

Preliminary Data: To define parental interests and attitudes regarding prospective follow-up of minor aged daughters, we recently completed a qualitative pilot study, involving semi-structured telephone interviews of 47 adult female OFBCR participants with a prior breast cancer. Findings illustrate high parental acceptance. Mothers also provided invaluable, practical advice that we have integrated into the design of all recruitment materials, staffing considerations, consenting process, study flow and setting for the planned study.

Objectives: 1) To identify motivating factors for youth study participation; 2) To assess girls' level of acceptance/dissatisfaction across data collection measures; 3) To assess girls' interest in future participation in a youth cohort; 4) To learn ways to improve future youth study design, recruitment and retention.

Methods: Semi-structured interviews will be audiotaped and transcribed verbatim. Transcript analysis will be based on grounded theory and continue until saturation within categories is reached. ATLAS/ti software will assist categorization of identified codes and themes.

Conclusions: Asking young participants to share their experiences and attitudes about study participation in their own words will hopefully yield deeper appreciation of their willingness to further participate in a large-scale study of the genetic and environmental determinants of intermediate outcomes of breast cancer and how best to improve its future design.

I AM THE CURE.™ in Greater Cincinnati

Tracy L. Hawkins
Gtr. Cincinnati Affiliate of Susan G. Komen for the Cure

The 2007 Greater Cincinnati Race for the Cure included Komen's new I AM THE CURE.™ program, educating the public about the importance of a positive breast health program and empowering people to take charge of their own breast health. I AM THE CURE.™ was created when the 2006 national survey of Race participants found that while 71% could recall something about breast health, breast cancer or the Race itself two days later, only 30% could recall health information and the 18% who remembered the statistics were confused about the specifics and spoke in generalities!

The goal of I AM THE CURE.™ is to teach that:

- The greatest risk factors are being female and growing older;
- It knows no boundaries;
- The key to survival is early detection;
- One in eight women will be diagnosed in their lifetime;
- Three simple steps for early detection are regular mammograms, clinical exams and breast self-exams;
- It is important to make healthy lifestyle choices; and
- It affects more than just the patient – friends and family need support too.

Greater Cincinnati's program had approximately 200 NEW community volunteers divided into one of three groups:

CURELEADERS. High school cheerleaders communicated simple, action-oriented messages for participants: (1) **Yearly check-ups can save lives** - the key to survival is early detection; (2) **Every person, everywhere** - breast cancer knows no boundaries, be it age, gender, socio-economic status or geographic location; (3) **Best protection is early detection** - regular mammograms, clinical exams, monthly self breast exams help reduce risk; and (4) **Raise your voices for healthy choices** - it is important to make healthy lifestyle choices.

ROVERS. UC's College of Nursing's honor students and faculty, and staff from area breast cancer organizations infiltrated thousands of race-day participants to educate about breast cancer and share the I AM THE CURE.™ messages.

SURVIVORS & CO-SURVIVORS. Survivors, their families and friends handed out hundreds of special keychains, each with tags in varying sizes demonstrating the average size of tumors found by (1) regular mammograms, (2) first mammograms, (3) monthly self-exams, and (4) accident.

Initial feedback indicates that I AM THE CURE.™ was well received by all generations at the Race and that the simple messaging was effective in educating and empowering the public. The most common reaction to I AM THE CURE.™ has been a request to incorporate it into more community activities!

Early Exposure to Dietary Bisphenol A Alters the Mammary Proteome in Adult Rats

Sarah Jenkins, Nandini Raghuraman, and Coral Lamartiniere, University of Alabama at Birmingham, Department of Pharmacology & Toxicology

The environmental endocrine disruptor, bisphenol A (BPA), has recently received media and legislative attention for its potential role in various human health maladies. Studies have shown BPA exposure to be ubiquitous, as it is used to manufacture polycarbonate plastic and epoxy resin found in a plethora of consumer goods. Levels of BPA have been found in adult serum, fetal plasma, urine of children and adults, amniotic fluid, breast milk and colostrum, breast adipose tissue, and placental tissue. This has provided ample evidence for widespread BPA exposure to humans, from the developing fetus to adults. However, the period of time prior to the onset of puberty appears to be a critical window of development that can be altered by exposure to certain chemicals, leaving permanent effects even in the absence of the original effecter. We have previously shown that when coupling prepubertal exposure to BPA with a dimethylbenz[a]anthracene model of rodent mammary carcinogenesis, females exposed to BPA developed a significantly greater number of mammary tumors than control females. To elucidate the molecular mechanisms of this effect, we have employed a combination of proteomic technology and traditional immunologic techniques. Lactating dams were gavaged with 250 µg BPA/kg body weight/day or an equal volume of sesame oil. Female offspring were exposed to BPA indirectly through lactation from postpartum day two until 20. At 50 days postpartum, we found a significant increase in cell proliferation and a significant decrease in apoptosis in the terminal end buds of BPA exposed females. Additionally, the mammary glands of BPA exposed females were shown to down-regulate Raf kinase inhibitor protein (RKIP), DJ-1, and gamma synuclein. Of particular interest is the down-regulation of RKIP, which serves to control activation of the protein kinase Raf. Studies using cell culture and/or animal models have shown a down-regulation of RKIP to be associated with cancer development. A recent examination of 103 breast cancer tumor specimens found an inverse correlation between RKIP expression and the incidence of metastasis. Collectively, these results suggest that even low levels of BPA exposure during critical windows of development are sufficient to induce deleterious changes in protein signaling with in the mammary gland that may play a role in a greater susceptibility to mammary carcinogenesis during adulthood.

Hormonal regulation of PR isoform expression during development: relationship to proliferation in rat mammary gland

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Department of Physiology, Breast Cancer and the Environment Research Center, Michigan State University, East Lansing, MI

Progesterone (P) is important for normal mammary gland development and is implicated in increasing breast cancer risk in women receiving combined estrogen (E) plus P menopausal hormone therapy. P renders its biological effects through progesterone receptor (PR) isoforms A and B. The objective of the current study was to investigate the effects of the ovarian hormones E and P on PR isoform expression and the relationship of PR isoforms to proliferation during different mammary gland development stages in the rat. Sprague-Dawley rats were bilaterally ovariectomized at 5 weeks of age, before onset of puberty, or at 18 weeks of age, after mammary gland maturation was completed. The rats were then injected *s.c.* with exogenous E (17 µg/kg body weight), P (15 mg/kg), a combination of E and P, or vehicle control for 3 consecutive days. Ovary intact control animals were sham operated and injected with vehicle (olive oil). In both the pubertal and the adult animals, ovariectomy (OVX) resulted in loss of PRA expression. Treatment with E alone restored PRA expression. PRB expression was not changed by OVX or hormone treatments. Proliferation was significantly reduced by OVX. Treatment with P had a slight positive effect on proliferation in the pubertal gland, but not in the adult gland. E alone induced proliferation in the pubertal gland and no additional effect was observed with E+P. In the adult, E treatment had only a slight effect on proliferation, whereas E+P had a highly synergistic effect on proliferation. At both developmental stages, most proliferation occurred in PRB+ cells, whereas PRA+ cells rarely proliferated. In summary, mammary proliferation in pubertal rats was highly sensitive to E, whereas in the adult gland the greatest proliferation was observed with E + P. However, P alone did not induce proliferation in the adult gland. This suggests that in the adult, E-induced PRA expression is necessary for P to induce proliferation in PRB+ cells. This raises the possibility that P induces a paracrine factor in PRA+ cells that is required for proliferation in PRB+ cells. While E alone has little mitogenic activity it acts permissively to promote P induced proliferation through up-regulation of PRA expression.

The role of progesterone receptor A in mammary gland development and mammary cancerogenesis in the rat

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Epidemiological studies have implicated progesterone (P) in increasing the breast cancer risk in women receiving combined estrogen (E) and P hormone therapy. In the breast, P acts through two progesterone receptor (PR) isoforms. In the normal human adult breast, PRA and PRB are expressed in equimolar ratios, whereas in many breast cancers PRA expression is predominant, which is associated with a poor prognosis for survival. We hypothesize that a subpopulation of PRA positive (PRA+) cells has mammary stem/progenitor cell characteristics and is directly involved in mammary cancer development. To study mammary stem cells *in situ*, Sparague-Dawley rats were labeled with BrdU (70 μg/kg body weight, 2 injections 24 hrs apart) at 21 days of age, a period of active growth of the mammary epithelium. Label-retaining cells (LRC) were detected in adult glands 18 weeks later. Analysis of LRC, some of which are putative stem cells, revealed that more than 60 % of LRC were PRA+ and a significant proportion of PRA+ LRC were negative for differentiation markers PRB and STAT5a. We hypothesize the subpopulation of PRA+PRB-Stat5a- LRC may be stem/progenitor cells in the rat. In a second experiment, mammary cancers were induced with DMBA (100 mg/kg body weight) given intragastrally at 50 days of age. Analysis of PRA expression in tumors revealed a dramatic increase in the percentage of PRA+ cells (more than 50%), whereas in adjacent non-tumorous tissue PRA expression was lower and comparable to the levels in the adult virgin gland. Conversely, the percentage of PRB+ cells in tumors decreased and the percentage of PRA+PRB+ cells was significantly lower than in normal glands. The proliferation rate in tumors was significantly higher than in adjacent normal tissue, and a large percentage of proliferating cells were PRA+. Of the proliferating PRA+ cells, 66% were PRB-Stat5a-. Additionally, some PRA+ cells acquired expression of TERT, a main determinant of telomerase activity. Telomerase activity is a putative marker of stem cells or cancer stem cells. In summary, our results suggest that the subpopulation of PRA+PRB-Stat5a- cells, putative mammary stem/progenitor cells, is markedly expanded in mammary cancers. We speculate that PRA+PRB-STAT5a- cells may be a primary target for mammary cancer initiation.

Radiation increases the outgrowth of human mammary epithelial cells with extended proliferative potential in serum-free cultures

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We are using cultured human mammary epithelial cells (HMEC) to evaluate the potential of a prototypical environmental stressor, ionizing radiation (IR), to cause pre-malignancy-associated epigenetic changes. To date, few studies of environmental stressors have focused on their effects on epigenetic phenomena such as gene methylation. Locus specific hypermethylation has been associated with abrogation of tumor suppressor gene expression in breast cancers. In serum-free growth medium, HMEC from histologically normal breast tissue growth arrest after 5-20 population doublings, exhibiting senescent morphologies and elevated expression of the cyclin-dependent kinase inhibitor $p16^{INK4a}$ - a tumor suppressor. This p16-dependent "stasis" involves activation of another well-known tumor suppressor - pRB. Variable p16 expression is also observed in epithelial cells *in situ*, suggesting that the conditions that induce it may have physiologic relevance. In HMEC cultures from normal tissues, a rare variant p16(-) cell population that is capable of long-term growth (50-100 PD total) arises spontaneously with frequencies between $1x10^{-5}$ and $1x10^{-8}$, depending on specimen and culture conditions. We sought to determine whether moderate doses of radiation would alter long-term growth potential, p16 expression, and p16 promoter methylation in HMEC. We found through replicate experiments with HMEC that 2 Gy X-irradiation causes an increase in the appearance of p16(-) cells with long term growth potential. Flow cytometry confirmed that differences in BrdU incorporation - a measure of growth rate, in the treated and untreated populations were statistically significant (P = 0.0016). The effects of different doses of radiation as well as radiation quality could be distinguished by flow cytometric analysis of BrdU incorporation and senescence associated beta-galactosidase activity - a measure of p16-dependent stasis. We hypothesize that radiation, possibly acting through the generation of reactive oxygen species, causes epigenetic changes in p16 expression, resulting in extended proliferative potential – a necessary step in the development of malignancy. Our studies will lead to the development of a better scientific basis for understanding exposures and risks to humans from ionizing radiation and other environmental stressors.

Optical Reflectance Spectroscopy: A safe new method that quantitatively measures breast tissue properties during puberty for scientific studies.

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Background: In adult women, mammographic density is strongly associated with breast cancer risk, and is determined by genetic and environmental factors. Most current studies of the determinants of mammographic density are conducted among adults, yet much of the development of breast tissue occurs during puberty and adolescence, a time when breast tissue may be particularly sensitive to environmental exposures.

Pubertal breast development is characterized usually by breast Tanner Staging (TS). One of the primary limitations of TS is that the categories are based on anatomical features of the breast. Methods that measure different tissues present in the breast such as mammography, MRI, and DEXA have limitations, including cost and exposure to ionizing radiation. A novel method that overcomes these limitations is optical reflectance spectroscopy (OS) which uses near infrared visible light reflected off the breast. The resulting spectral variation is captured using principal components analysis and individual scores are generated.

We are conducting a pilot study in 300 girls to determine whether OS can be used in a large-scale study of the genetic and environmental determinants of breast tissue development and other characteristics among girls with a family history of breast cancer.

Hypothesis: Measures of breast tissue characteristics obtained from OS correlate with Tanner Stage and hormones of the GH-IGF1 axis.

Objectives: 1. To measure breast development in girls aged 7-17 years using TS and OS. 2. To measure hormones of the GH-IGF1 axis to assess associations with measures of breast tissue obtained from OS.

Methods: A cross-section of girls aged 7-17 years who have a parent participating in the Ontario Familial Breast Cancer Registry will be invited to participate. Epidemiologic data, blood samples, OS and TS will be obtained at a clinic visit. Correlation and regression analysis will be used to examine the relationship between the OS measures, TS, and hormone levels.

Results: A small feasibility study conducted among girls aged 10-14 years showed that the principal components representing lipid and water content of the cells correlate with breast tanner stage.

Conclusions: The results of this study will show whether OS is a useful tool for quantitatively measuring breast tissue development and characteristics in scientific studies of adolescent females.

Annotated Bibliography of Relevant Journals for Possible Publication of Advocate and Communication Research

Samantha Munday, Carolyn LaPlante, Sandi W. Smith, Charles K. Atkin

Institutions: Samantha Munday is a graduate student and Carolyn LaPlante is an undergraduate student at Michigan State University where Sandi W. Smith and Charles K. Atkin are Professors.

Background: Dissemination of information from research and outreach is paramount. Finding the journal which best addresses a given target audience for the results of a study or outreach effort is a critical part of this process.

Objective: The objective of this research was to help breast cancer advocates and communication researchers find the right journals for which to submit their work for publication.

Methods: Two searches were completed in the search engine Web of Science. One search used the keywords “breast cancer communication” and the second used the keywords “breast cancer media.” These two searches found relevant articles of interest and their journals. Each article, along with the journal in which it was published, was placed into four non-mutually exclusive categories to describe the type(s) of communication they addressed: mass media, new media, interpersonal, and/or doctor-patient. After the search, two more journals were added to the list upon the advice of other experts in the medical and dietetic fields. Subsequently, the style format, purpose, audience, author types, communication type(s), space limitations, and a link to the submission guidelines for authors from each journal are included in the resulting document.

Results & Conclusions: A total of 30 possible journals for breast cancer advocacy and communication research were documented in this way and included in an annotated bibliography. Five of the journals researched are highlighted in the poster, but all are included in the handout that will be available from the researchers and online at http://bcerc.msu.edu/.

Serotonin system as a novel biomarker for breast cancer

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Pregnancy associated breast cancers have the worst prognosis along with high mortality rate. However early full term pregnancy is protective against breast cancer (Polyak, 2006). The factors responsible for this paradoxical association between pregnancy and breast cancer are unknown. However it is known that majority of breast cancers originate from the mammary epithelial cells (Howard and Ashworth, 2006; Polyak, 2006). Hence it is very important to know and understand the systems that are present in the mammary epithelial cells but are altered in the transformed cells making them cancerous and metastatic. Previously our lab has reported the presence of serotonin (5-HT) system in the mammary epithelial cells where it functions (autocrine-paracrine manner) as a crucial regulator of mammary gland homeostasis especially in the lactation-involution switch (Matsuda and Imaoka, et al, 2004, Stull and Paiv et al, 2007 (PNAS in press)). Other studies have shown that the involution microenvironment is promotional for breast cancer migration and metastasis (Kwak and Gustafson, et al, 2006; McDaniel and Rumer, et al, 2006; Schedin, 2006). Hence in this study we explore the 5-HT system in breast cancer cells in comparison to mammary epithelial cells. Tryptophan hydroxylase 1 (TPH1), the rate limiting enzyme for serotonin biosynthesis, is found to be highly up regulated at both the mRNA and protein level in the breast cancer cells indicating their high serotonin synthesis capability. Although there is no change in the levels of serotonin reuptake transporter (SERT), the serotonin receptor expression (5-HTR7) is altered in the breast cancer cells. Correspondingly the signaling downstream of the 5-HT receptor is also found to be altered. Also 5-HT is growth inhibitory for the mammary epithelial cells however the breast cancer cells are refractory to this growth inhibition by 5-HT probably due to altered 5-HT system. Thus 5-HTsystem is a novel biomarker for breast cancer which warrants further investigation and might provide new therapeutic targets.
Key words: Serotonin (5-HT), Tryptophan hydroxylase 1 (TPH1), Serotonin reuptake transporter (SERT), lactation-involution switch

Exposure to bisphenol A (BPA) during the prenatal or prepubertal period induces a similar genomic signature at the time of high susceptibility of mammary carcinogenesis.

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Bisphenol A (BPA) is a monomer used to manufacture polycarbonate plastic and the resin used for most food and beverage can liners, and can act as an endocrine disruptor. In this study, we have analyzed prenatal or prepubertal exposure to BPA on the genomic signature of rat mammary glands. Pregnant Sprague Dawley rats received, by gavage, 25 or 250 μg BPA/kg body weight (low and high dose groups) or an equivalent volume of sesame oil (control group) from the tenth day post-conception to parturition for prenatal exposure. For prepubertal exposure, nursing dams received 250μg BPA/kg BW from parturition to weaning. When the offspring reached 50 days of age, 10 female offspring from each group were sacrificed and RNA was extracted from the mammary glands for screening of genomic signature using Agilent platform of 60-mer oligo-microarrays containing 22,000 features. We have identified 1246 genes modulated by BPA treatment, with q-value less than 0.05. Cluster analysis showed that 2 gene ontologies (nucleic acid binding and chromosome) were upregulated in all groups and 4 gene ontology groups, anatomical structure morphogenesis, basement membrane, collagen and extracellular matrix are common among the downregulated genes of the 3 modalities of treatment. Through Ingenuity Pathways Analysis, we have found that estrogen receptor and aryl hydrocarbon receptor signaling pathways were the canonical pathways overrepresented among the upregulated genes. Some of the genes were common for both pathways, such as EP300, SRC and NCOA2. Others, as GRB2 and MAP2K1 are part of the estrogen pathway and, AHR, CDKN1A, TP53 exclusive for AHR pathway. Among the downregulated genes we found GAD1, RHOA, AKT, IGF1; the latter two are part of axonal guidance signaling pathway. Our data led us to conclude that BPA induces the same genomic signature independent of time of exposure. Most importantly, the data indicate that changes occur at the age in which the mammary gland presents the highest susceptibility to chemically induced carcinogenesis. (This work was supported by NCI and NIEHS Grant UO1 ES012771).

Breast cancer incidence is related to uranium particulate exposure in a population living near a uranium refinery.

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Background: Ionizing radiation exposure has long been recognized as a risk factor for breast cancer in humans, probably as a result of the induction of DNA double-strand breaks. Recently, studies of mammary cell lines have noted that the heavy metals cadmium and uranium seem to exhibit estrogenic activity. The elevation in breast cancer mortality in populations living around uranium mines or uranium fuel processing facilities may be related to both the heavy metal and radiation effects inherent with uranium exposure.

Objective: To determine if breast cancer incidence is related to exposure to uranium (U) and radiation in a population residing around the Feed Materials Production Center (FMPC), a uranium refinery in Fernald, Ohio.

Materials and Methods: The FMPC was part of the US Department of Energy (DOE) nuclear weapons complex, and produced uranium metal products during operation from 1952-1989. Releases from the site resulted in exposure to soluble and insoluble forms of uranium, and ionizing radiation. The Centers for Disease Control and Prevention (CDC) developed dose estimation models for estimating exposures to individuals who lived within the exposure assessment domain (area within a five mile radius from the perimeter of the plant). We used these algorithms to estimate exposure for 8770 persons enrolled in the Fernald Medical Monitoring Program (FMMP), including 4823 women. During the 17 year tenure of the FMMP we have tracked breast cancer incidence, verified with pathology reports. We used the 174 incident breast cancer cases, diagnosed since 1990, in logistic regression analyses with appropriate covariates (age, family history of breast cancer, parity, age at first birth) to determine if there was a relationship between uranium/radiation exposure and breast cancer incidence.

Results: The mean value [sd] of the uranium particulate exposure metric for women in the three exposure groups was 0.086[0.069], 0.354[0.069], 1.596[1.098], and for cases was 0.738 vs. 0.614 for controls. When exposure was categorized in three groups, the risk of breast cancer in the highest exposure group (N=1568), compared to baseline (N=2515), was significantly elevated (OR=1.62, 95% confidence interval 1.15, 2.30), with only age and exposure in the model. In the final multivariate model, the OR for exposure was 1.71 (95% CI 1.21, 2.43), and, with age forced in the model (OR=0.98), both post-menopausal status (OR= 3.87) and number of relatives with breast cancer (OR=1.39) were retained as significant factors.

Conclusions: For women living within five miles of a uranium processing plant, degree of exposure to uranium particulates was related to risk of incident breast cancer.

Pilot Study of Serum Biomarkers of Polyfluoroalkyl Compounds in Young Girls

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Background: Polyfluoroalkyl compounds (PFCs) and their salts, such as perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), are chemicals that have wide consumer and industrial applications and known environmental persistence. PFCs have been detected in humans and wildlife, and health effects have been noted in laboratory animals, including changes in mammary gland structure and function.

Objective/Hypothesis: Within the NIH Breast Cancer and the Environment Research Centers (BCERC), we conducted a pilot study of multiple environmental biomarkers in young girls (age 6-8 years), including PFCs, followed by a second study at the Ohio site where elevated levels of PFOA had been detected in the pilot study.

Methods: Participants for the pilot study were recruited from area schools in Cincinnati and Northern Kentucky (n=27) and membership of the Kaiser Permanente health maintenance organization in the San Francisco Bay area (N=28). Blood was collected using a standard protocol and materials provided by the Centers for Disease Control and Prevention (CDC), and assayed for the perfluoroalkyl acids using high-performance liquid chromatography-tandem mass spectrometry.

Results: Four of the seven PFCs, including PFOA and PFOS, were detected in all samples, and only one was detected in less than 70%. The median values for PFOA differed by site (PFOA -12.9 ng/ml for California and 20.2 ng/ml for Greater Cincinnati), an unexpected finding. Within the Ohio site, 14 of the 15 girls in one community had PFOA values above the NHANES 1999-2000 95th percentile value for children 12-19 years (11.2 ng/ml, Calafat, 2007). In the follow-up study of 42 girls from the community with higher values, the elevation in serum PFOA persisted (median 17.4 ng/ml, range 6.9-42.6 ng/ml serum), with 31 having values above the NHANES 95th percentile. For the subset of girls from greater Cincinnati who were in both the pilot and second study, the difference between PFOA measures for each girl, one year apart, was a mean decrease of 10.2 ng/ml for girls in the community with the higher values ($p<0.0001$ under a one sample t-test with $H_o=0$) and a mean decrease of 1.8 ng/ml ($p<0.001$) for the girls from the community with the lower values.

Conclusions: Sufficient between-person variation in PFC levels exists to enable an investigation of association with age of onset of pubertal maturation. The elevated

serum PFOA levels in one community in the greater Cincinnati areas appear to be decreasing, but cannot be linked to a source at this time. Further research is required to identify the source and potential health effects. *The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy.*

The Role of Endocrine Disrupters in Breast Cancer
Tehreem Rehman, Huntington Breast Cancer Action Coalition
Heidi Park, Great Neck Breast Cancer Coalition

In an effort to raise awareness among youth, the Great Neck and Huntington Breast Cancer Coalitions sent students Heidi Park and Tehreem Rehman to the Ana M. Soto Laboratory and the Fox Chase Center to learn about the effects of the environment on breast cancer, particularly endocrine disruptors Bisphenol-A (BPA) and dioxin. Both BPA and dioxin are suspected to have contributed to the increase in breast cancer among women in the United States for the past several decades. It is suspected that the reason behind this and the increased incidence of precocious puberty among young girls is long-term exposure to these hormone-mimicking or endocrine-disrupting compounds (EDCs). Many scientists conjecture that EDCs can transiently or permanently affect mammary gland development depending on dose, length of exposure, and whether or not the exposure took place during a critical period of mammary gland growth or differentiation. Studies done by the Ana M. Soto Laboratory and the Fox Chase Cancer Center, as well as others, have linked fetal exposure to these two chemicals to abnormal mammary gland development and mammary gland tumors. This is a summary of what they have learned and their experiences at their respective laboratories.

Evaluation of Breast-Tissue Estradiol and Triglycerides in a High-Risk Cohort
Craig Rowell, Siya Lem, Sarah Kim, Mark Carpenter, Victoria Scott, Victoria Seewaldt
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Using patient-matched serum and extracellular fluid from radom periareolar fine needle aspiration samples (RPFNA-EF), obtained from women at high-risk for developing breast cancer, we evaluated estradiol (E2) and triglyceride (TG) levels. We compared these values to the menopausal status, BMI and Masood cytology index values for each patient. In general, the estradiol levels of the RPFNA-ER compartment we 3X that observed in the serum. There were no statistical differences in the tissue level estradiol concentrations based on menopausal status. There was a slight, but significant, negative correlation between BMI and RPFNA estradiol/TG levels. Tissue level estradiol levels were associated with increasing Masood cytology index.

Isolation and differentiation of adipose-derived adult stem cells from the mammary gland.

Craig Rowell and Victoria Seewaldt
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The potential role of stem cells and/or progenitor cells in cancer development continues to gain interest. Recent evaluations have pointed to an important source of progenitor cells; Adipose Derived Adult Stem cells (hADAS). The differentiation potential of these cells has been demonstrated by their conversion to phenotypes including adipocytes, chondrocytes, osteoblasts and others. The differentiation into an osteoblastic form is of particular interest given the metastatic potential of breast cancer to bone. In this pilot project we demonstrate that hADAS cells derived from the mammary gland can differentiate to osteoblast-like cells. Briefly, hADAS cells were separated from tissue derived from a patient undergoing prophylactic mastectomy. Isolated cells were cultured in the presence of ascorbate, B-glycerophosphate, dexamethasome and 1,25 vitamin D. Initial assessment of conversion was evaluated with alizarin red staining, indicating the presence of calcium deposition by osteogenic cells. Future evaluations will focus on more stringent evaluations of osteogenic activity as well as potential use of this cell type for creating models for investigation of metastasis.

Dietary Restriction, Meal Anticipation, and Mammary Tumor Formation

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Dietary/energy restriction (DER) is arguably the most potent approach to the prevention of experimentally induced breast cancer that has been identified, to date. To reduce calories in experimental animals, the availability of food is restricted, inducing a “meal fed” vs. an eating at will or ad libitum state. It is not known whether the preventative effects of DER are due to calories or meal feeding, and if the latter, by what mechanism. Like humans, animals learn to anticipate when meals will occur and secrete hormones prior to meals allowing them to digest and metabolize nutrients more efficiently. Our hypothesis is that the hormonal events due to meal anticipation confer protection from cancer. We examined these anticipatory responses in female rats fed on a predictable or unpredictable schedule, compared to animals fed ad lib. Since a large meal causes many of the same stress responses as other forms of stress, we measured their endogenous release of stress hormones. These studies will allow us to dissociate anticipatory nutritional effects from anticipatory stress effects associated with meal feeding. Our data suggest meal fed animals weigh significantly less than ad libitum fed animals, and chronic restriction influences the release of metabolic hormones prior to meals in animals which can predict the timing of the meals. Additionally, we have profound effects on the size and morphology of the mammary gland which may afford protection to carcinogenesis.

Key Words: Dietary/energy restriction (DER), meal feeding, stress, meal anticipation

Persuading Mothers to Perform Breast Cancer Prevention Practices with their Pre-adolescent Daughters: A Pilot Message Study

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Background: Breast cancer prevention messages have traditionally targeted the behavior of adult females, recommending regular mammograms, breast self exams, and adopting a healthy lifestyle as key message components. Recent research has extended the prevention interest to mothers of pre-adolescent girls because those who are overweight are more likely to experience puberty early, which increases their later risk for breast cancer. Additionally, certain chemical exposures may also impact their later breast cancer risk. As a result, early action is recommended for young girls. The need for early action also echoes the sentiment of the precautionary principle, which holds that it is always wise to take precautionary actions to prevent harmful consequences in advance, even in the absence of explicit evidence for such consequences. However, as young girls are not equipped with enough resources to reduce breast cancer risk themselves, mothers play an important role in influencing lifestyle factors of their daughters.
Objective: The current study focuses on women's perceptions of breast cancer prevention messages. We examine whether message source, message type, and involvement influences mothers' evaluations of health messages, behavioral intentions to adopt recommended actions, and beliefs about the effectiveness of recommended actions in reducing breast cancer risk among pre-adolescent females.
Method: Approximately 50 women participated in this pilot study with a 2 (message source: researcher vs. physician) x 2 (involvement: high vs. low) x 3 (message type: lifestyle vs. chemical vs. developmental) factorial design. Participants accessed a Website where the online experiment was posted and were randomly assigned to one of 12 experimental message conditions. Participants read one high- or low-involvement lifestyle, chemical, or developmental message about breast cancer prevention that is authored by a physician or a researcher. After reading the message, participants completed a post-test survey with items that measure message perceptions, attitude, self and response efficacy, and behavioral intention to engage in recommended behaviors.
Results/Conclusions: Mean item scores per condition were analyzed using analysis of variance (ANOVA) techniques. As predicted, women with daughters were more likely to have positive attitudes and intentions to engage in breast cancer prevention recommendations. Implications for future message design targeted toward our population of interest – specifically pertaining to message source, involvement, and type – are discussed.

The Types and Sources of Meaningful Messages about Breast Cancer

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Background: *M*eaningful message research examines interpersonal messages "…remembered for extremely long periods of time and which people perceive as a major influence on the course of their lives" (Knapp, Stohl, & Reardon (1981, p. 27). Meaningful messages were further studied by Smith and Ellis (2001; 2004; Smith Ellis, & Yoo, 2001), who showed that they are guides to action. *Objective:* Meaningful messages can aid breast cancer innovative outreach efforts by informing researchers about the types and sources of messages women are recalling that may lead them to, or deter them from, action.
Methods*:* In this study, N=137 women were recruited to take an online survey regarding meaningful messages they recalled about breast cancer. A total of N=77 women recalled such a message, described it, and identified its source.
Results*:* Four categories and 16 subcategories of message types emerged: (1) breast cancer awareness (statistics/facts, breast cancer affects us all, campaigns/organizations), (2) prevention (health/be proactive, improve/change a health behavior, family risk), (3) detection (breast self exam, mammograms, breast self exam and mammograms, early detection), and (4) treatment (appearance, choices, survival choices, social support, womanhood, fear/pain/negative, appreciation for everyday life). The analysis shows that 39% recalled a detection message, followed by 30% for treatment, 29% for awareness, and 2% for prevention.

Four categories of sources emerged accounting for 94% of all sources reported. These sources are family members, media, doctors/health care professionals, and friends. Media constituted the most reported source across all four categories of meaningful messages (30%), followed by family members (25%), friends (22%), and doctors (17%).

The majority of participants who had an awareness message (22 total) reported its source as the media (11), followed by friend (4), family member (3), doctor/health care professional (3), and other (1). Two participants' messages were about prevention, one each from a family member and the media. In the detection category, (30 total) family members (9) were reported most often, closely followed by doctors/health care professionals (8), media (6), friends (5), and other (2). Treatment meaningful messages (23 total) came mostly from friends (8), then family members (6), media (5), doctor/health care professionals (2), and other (2).
Conclusions: Respondents recalled few messages about breast cancer prevention, but they did recall messages about detection. This research demonstrates the power of the media, as well as the possibility that messages from family and friends are more likely to impact women's lives than those from the medical community.

Neonatal Diethylstilbestrol (DES) or Genistein Exposure to Uterine Cancer Risk in Adult: Putative Epigenetic Regulation of *Inhibitor of DNA binding protein 3* (ID3) in Mouse Uterus.

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Background: Early life exposure to estrogenic endocrine disruptors like disethylstilbestrol (DES, a synthetic estrogen) or genistein (GEN, a phytoestrogen) in early life may induce uterine cancer in humans and rodents in adult life. Previously, we reported that neonatal exposure to DES or GEN on Day 1-5 induced uterine adenocarcinoma by 18 months in a CD-1 mouse model. We hypothesize that neonatal exposure to those compounds increases later cancer risk by epigenetic modulation via DNA methylation of specific genes.

Objectives: In this study, we investigate the effects of neonatal exposure of DES or estrogenic equivalent doses of GEN in altering DNA methylation of specific genes, as a molecular mechanism underpinning early life environmental exposures as a modifier of carcinogenesis in later life.

Methods: Four groups of neonatal mice were treated with 1 or 1000 µg/kg DES, 50 mg/kg GEN or corn oil vehicle at days 1-5. Mice were sacrificed on day 19 before puberty, at 6 months and at 18 months. Half of the 6 months and 18 months groups were ovariectomized 7 days before sacrifice, while half of them remained intact. Differential methylation patterns of the different treatment groups were determined by Methylation Sensitive Restriction Fingerprinting (MSRF) analysis using uterine DNA obtained from 6-month old ovariectomized or intact mice exposed neonatally to either DES or genistein. Thirty-two clones were identified, and we found homology to known genes in the NCBI or UCSC database.

Results: An aberrant transcript expression and methylation pattern of *Inhibitor of DNA binding protein 3* (ID3) was identified to be differentially methylated upon neonatal exposure to DES and GEN compared to the untreated control. ID3 encodes proteins that control cell differentiation and proliferation by interfering with binding of DNA to transcriptional factors; it is regulated by vascular epithelial growth factor (VEGF). ID3 deregulation has been reported in various tumors. To our knowledge, this is the first time the altered transcript expression and methylation status of ID3 promoter sequence in the uteri of mice neonatally exposed to DES or genistein has been reported. These animals have been shown to develop cancers from exposure to neonatal estrogenic endocrine disruptors.

Conclusions: Our study supports the hypothesis that a set of genes (an epigenome) could be reprogrammed by estrogenic compounds during critical periods of estrogen end organ development, a change that may underpin enhanced propensity for carcinogenesis.

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High Fat Diet and Elevated Body Weight in C57BL/6 Mice Impacts Pubertal Mammary Gland Development.

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Obesity is thought to accelerate puberty and breast development in girls. During puberty, the breast epithelium is highly proliferative and alterations affecting the rate of development of the gland may increase or decrease breast cancer risk. To better understand how elevated body weight may alter mammary gland development, 3-week-old C57BL/6 female mice were fed for 4 weeks with control diet (CD) (10% kcal fat, 70% kcal carbohydrate, 20% kcal protein) or high fat diet (HFD) (60% kcal fat, 20% kcal carbohydrate, 20% kcal protein). The HFD resulted in increased body weight, mild glucose intolerance and increased plasma insulin and leptin. The HFD group had larger mammary fat pads and larger epithelial area. Additionally, ducts in HFD mice penetrated the fat pad further and had fewer and smaller terminal end buds (TEB). Immunohistochemistry showed that the percentage of estrogen receptor alpha (ERα) positive and progesterone receptor A (PRA) positive cells was lower in ducts of HFD mice. While there was no difference in the percentage of proliferating ductal cells between the diets, there were more proliferating cells in TEB of CD mice. Another group of mice fed the same diets were ovariextomized (OVX) at 7 weeks of age. Then, at 11 weeks of age, they were treated for 5 days with estrogen (E) and/or progesterone (P). Ovariectomy itself increased body weight, which was greatly enhanced by HFD. Both insulin and leptin levels were increased in OVX+HFD mice. Mammary fat pad area was larger in OVX+HFD mice, independent of E and/or P treatment. Although differences in mammary gland morphology in OVX hormone-treated CD and HFD mice were not significant, hormone treatments increased epithelial area on CD but not HFD mice. IGF-I levels were dramatically increased in OVX+HFD mice treated with E+P. In summary, HFD during puberty increased body weight and levels of insulin and leptin, which are known mitogens for mammary epithelial cells. Notably, OVX+HFD increased growth of the mammary epithelium and adipose stroma and desensitized the mammary epithelium to E and/or P-induced proliferation. HFD modulated ERα and PRA expression and hormone responsiveness, which might alter the susceptibility of the epithelium to future initiating events involved in breast cancer.

The Falling Age of Puberty in U.S. Girls: What We Know, What We Need to Know

By Sandra Steingraber, Ph.D. Commissioned by Breast Cancer Fund
Poster Submitted by Brynn Taylor, Breast Cancer Fund

Background:
Girls today get their first periods, on average, a few months earlier than did girls 40 year ago, but they get their breasts one to two years earlier. Over the course of a few decades, the childhoods of U.S. girls have been significantly shortened. What does this mean for girls today and their health in the future? We know that early puberty is a risk factor for breast cancer and other mental and physical health problems. To better understand what's causing early puberty so that we can protect the health of our children now and as they age, Breast Cancer Fund commissioned the first comprehensive review of the scientific literature on the timing of puberty. In *The Falling Age of Puberty: What We Know, What We Need to Know* author, biologist, and cancer survivor Dr. Sandra Steingraber explores pubertal development and outlines nutritional, psychosocial and environmental factors that contribute to its timing.

Work Performed:
The report reviews the published literature in a wide range of fields—epidemiology, endocrinology, toxicology, evolutionary biology, sociology, child development, nutrition, veterinary medicine, media studies, anthropology—and describes the state of the evidence for each of the three categories of possible contributing factors: diet and nutrition; psychosocial factors; and environmental exposures to endocrine-disrupting chemicals. It then reviews what we know about the many negative psychosocial and physical health consequences of early puberty. To help reach a broader audience with this important information, the Breast Cancer Fund created an Advocate's Guide to highlight key findings and recommendations in an accessible format.

Conclusions:
The Falling Age of Puberty, a comprehensive review of the literature on the timing of puberty, revealed that there are areas of research where there is sufficient evidence for us to take public policy action now to protect the health of our girls in the future. Dr. Steingraber also highlights areas of research—in basic science of puberty, epidemiology, chemical testing, chemical tracking and biomonitoring—where more research is required. In the Advocate's Guide, Breast Cancer Fund outlines corporate accountability campaigns and legislative advocacy that will help address the possible causes of puberty and highlights what we can do personally and politically to address this disturbing trend.

Evaluation of the participatory research approach used by the Community Outreach and Translation Core (COTC) of the Bay Area Breast Cancer and the Environment Research Center (BABCERC)

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Background The commitments to community-based participatory research (CBPR) are central to the activities of the Community Outreach and Translation Core (COTC) of the Bay Area Breast Cancer and the Environment Research Center (BABCERC) led by Zero Breast Cancer. CBPR is a "*collaborative approach* to research that equitably involves all partners in the research process and recognizes the unique strengths that each brings". The BABCERC COTC primary aim is to ensure that the views and concerns of the breast cancer and environmental advocate community are heard by BABCERC researchers and that BABCERC research findings are disseminated to San Francisco Bay Area community members. The BABCERC COTC is one of four national Breast Cancer and the Environment Research Center (BCERC) COTCs.

Objective The purpose of this evaluation is to assess the extent to which BABCERC activities align with participatory research guidelines.

Methodology The evaluation proceeded in two stages. In the first stage, 12 in-person interviews were conducted with a representative group of the BABCERC community -- COTC members, researchers and community members and advocates. In advance of the interview, these participants completed a questionnaire, adapted from one validated by Green and colleagues for reviewers to rate the extent to which grant proposals adhere to the principles of participatory research. The second stage was a focus group with 2 researchers, 2 COTC members and 2 community members in order to hear participants' reactions to and feedback regarding stage one findings that had been shared with them in advance. All participants signed a consent form, and all data is reported in the aggregate with no reference to individual respondents. Standard qualitative techniques were used to analyze the interview and focus group data, clustering the data according to themes based on research questions and those emerging during the analysis. Quantitative results from the questionnaire data were reported in the aggregate.

Results and Conclusion Main findings from the interviews and focus group revealed that using a participatory research approach increases the learning and broadens the perspectives of all stakeholder groups, enriches data collected, and makes science more accessible to the public. The challenges of carrying out a participatory research project include differing expectations among stakeholders about roles and 'level of participation' in the project, under representation of some communities in the research, and the different languages and paradigms of stakeholders. These and other results will be shared and their implications for participatory research will be discussed.

Beaded ducts: mammary intraductal hyperplasias due to perinatal exposure to the xenoestrogen bisphenol-A

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Background: Lifetime estrogen exposure is considered the main risk factor for the development of breast cancer. Scientists have hypothesized that exposure to environmental estrogens could increase a woman's risk of developing breast cancer. Humans are routinely exposed to bisphenol-A (BPA), an estrogenic compound leaching from dental materials, food and beverage containers and other plastic consumer products. BPA has been detected in human plasma, amniotic fluid, placenta, umbilical cord blood, and breast milk. Perinatal BPA exposure is associated with significant alterations in mouse mammary glands that may translate into increased risk for mammary cancer including a heightened response to estrogen, increased ductal density, and an increase in the number of structures where cancers are thought to arise. Importantly, prenatal BPA exposure induces preneoplastic lesions, hyperplastic ducts and carcinomas *in situ* in rats. These structures have not yet been observed in BPA-exposed mice at any age. **Objectives:** To assess whether perinatal exposure to BPA increases the risk of developing mammary cancer in adulthood by examining mammary glands from BPA-exposed female mice for preneoplastic and neoplastic lesions. **Methods:** Pregnant mice were exposed to vehicle (50% DMSO) or a range of environmentally relevant doses of BPA from gestational day 8 through day 16 of lactation via subcutaneous osmotic pumps. At 3, 9, and 12-15 months of age, mammary glands were dissected and examined for the presence of epithelial alterations. Areas of interest were excised from whole mounts, sectioned, stained, and examined histologically. Lesions were characterized by immunohistochemistry. **Results & Conclusions:** BPA-exposed females had ducts with a "beaded" appearance that upon histopathological analysis were classified as intraductal hyperplasias. These preneoplastic lesions had a high proliferative index assessed by Ki67 staining. Remarkably, expression of smooth muscle actin, a marker for myoepithelial cells, was occasionally detected within the ductal lumen. Masson's trichrome staining revealed high content of fibrous collagen in the periductal stroma of most beaded ducts. Together, these results indicate that perinatal BPA exposure can induce mammary intraductal hyperplasias in adult mice, and "beaded" ducts may result from alterations in stroma-epithelial interactions.

The clock gene ARNT (Bmal1) and estrogen receptor Alpha are influenced by circadian disruption in rat mammary gland

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The circadian clock genes regulate daily oscillations in metabolism, hormone, and neurotransmitter levels. Aryl hydrocarbon receptor nuclear translocator-like (ARNT or Bmal1) is a clock gene that interacts with xenoestrogen and estrogen pathways, and expresses circadian rhythmicity in the Sprague Dawley rat mammary gland. Ligand-activated aryl hydrocarbon receptor (Ahr) or dioxin receptor heterodimerizes with ARNT, and initiates interaction with numerous dioxin-responsive genes such as Cytochrome P450 CYP1A1 and CYP1B1, which play a critical role in carcinogenesis. We hypothesized that disruption of the circadian rhythm induced by light could result in deregulation of basic processes such as cell cycle or cell proliferation. To test our hypothesis, we studied the expression of Estrogen receptor (ER) alpha, ER beta, ARNT and Ahr genes in young virgin female rats exposed to two different cycles of light for two weeks. A Control group was maintained under a standard 12 hour light/12 hour dark cycles, with lights turned on at 06:00h or zeitgeber time (ZT) 0 and off at 18:00h (ZT12). A Disrupted group was maintained under a standard cycle for 48 hours, thereafter, the light cycles were inverted for another 48 hours. Mammary glands were collected at six time points at four-hour intervals for RNA analysis by Real time RT-PCR. Both disrupted groups, ARNT and ER alpha, expressed a lower relative gene expression during the night cycle, at ZT18-22 and ZT14-18, respectively, when compared to the control. Instead, Ahr disrupted group exhibited a higher level of expression during the light cycle, at ZT2. ER beta did not exhibit any circadian rhythmicity in any of the groups. These results provide evidence that disruption of the circadian rhythm through light can alter the expression of important transcription factors and might contribute to a greater susceptibility to breast carcinogenesis. (Study supported by NIEHS grant UO1 ES012771 and grant supplement #16570)

Suggested Guidelines for Breast Cancer Websites

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Institutions: Pamela Whitten and Sandi W. Smith are both professors at Michigan State University where Samantha Munday is a graduate student and Carolyn LaPlante is an undergraduate student

Background: The Internet is a pervasively used tool for people seeking information about breast cancer. This past year a study was completed which included the evaluation of the basic design tenets and use of behavioral change theory components by the most hit 157 breast cancer websites. This study revealed many strengths and weaknesses of the most hit breast cancer websites.

Objective: To put forth a list of guidelines which breast cancer websites should strive to follow in order to improve their websites and have a greater impact on their audience.

Methods: Results from the study were reviewed and 18 findings were included in a list of guidelines. Nine of the findings regard basic tenet designs and nine of the findings regard behavioral change theory components. The nine guidelines from each category were chosen based on two qualities, the first being that the majority of the most hit 157 websites studied did not include the characteristic and, second that not having the characteristic on the website could have highly negative implications for users.

Results: The nine basic design tenet results chosen were: websites should list a date of last revision, include a webmaster, detail copyright restrictions, have equivalent text links for graphic links, avoid pop-up menus, create a help section, keep navigation bars consistent, fix dead links regularly, and have a site map.

The nine behavioral change theory components chosen were: websites should give a definition of breast cancer, include first and third person story telling about breast cancer, include role models for protective breast cancer behaviors and let users know they can be role models, add prompts to remind users to enact protective breast cancer behaviors, create in users the belief that they can perform breast cancer preventative behaviors and that those behaviors will be effective, make use of significant others, give social support, inform users about environmental risks, and tailor their websites to meet specific audience needs.

Conclusions: These 18 guidelines should be implemented on all breast cancer websites, as they are predicted to have positive effects on user's experiences.

Pilot Study of Urinary Biomarkers of Steroid Hormone Levels in the California Bay Area Breast Cancer and the Environment Research Center (BABCERC)

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Background: The BCERCs were initiated because of the association of early puberty (e.g. menarche) with increased risk of breast cancer, potentially due to longer exposure to endogenous estrogens. We are assessing puberty by breast and pubic hair development (Tanner staging), but direct measures of hormone levels would help elucidate endocrine control of reproductive maturation. Because hormone levels of cycling women vary over a monthly as well as a 24-hour period, integrated measures of urinary metabolites of estrogen and progesterone have been used to assess ovarian function. We conducted a pilot study using spot urine samples to examine the distribution of hormone metabolite levels in these young girls.

Hypothesis: Hormone levels will be higher in girls at later stages of breast development or with greater body fat than other girls, and will vary by other factors associated with puberty.

Methods: Part-way through recruitment of the baseline cohort in California, we aliquoted an additional spot urine specimen for measurement of the primary metabolites of estrogen (estrone conjugates or E1C) and a progesterone metabolite (abbreviated PdG), by enzyme-linked immunoassay. The distribution of these metabolites is examined, as well as differences by Tanner stage, body size, demographic variables and other factors, using creatinine-adjusted concentrations as both continuous and categorized variables.

Results: The 257 girls with hormones measured were 6 or 7 years old and had a similar racial distribution as the full cohort. Hormone metabolites were detected in all but one sample, and an additional 14 were very dilute and therefore excluded. Over 10% of these girls had E1C levels greater than the mean baseline levels (e.g. before ovulation) of cycling women, overlapping with mid-follicular phase levels, whereas PdG levels were consistently at or below those typically observed in the follicular phase. The mean and maximum hormone levels did not vary greatly by age or race, but African-Americans had the lowest values. The mean E1C was higher in the 28 girls who were Tanner stage 2 or greater, than in the remaining girls ($p<0.1$), but PdG was not. Further analyses will include additional and multiple factors.

Conclusion: Urinary hormone metabolites can be detected in young girls and appear associated with Tanner stage. The level of estrogen excretion detected several years prior to the first expected ovulation may indicate periods of unopposed estrogen stimulation. Collection of serial urine samples for measurement of day-to-day variation in hormone patterns is justified to capture peak levels and to better characterize the differences by various risk factors.

Methods Study of Assessing Mammographic Breast Density

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Background: Mammographic breast density is a strong predictor of breast cancer, influenced by hormonal environment and underlying genetics. Environmental factors, including endocrine disruptors, have the potential to interfere with the estrogen metabolism pathway thus causing changes in the levels of hormones which may lead to changes in breast density. **Objective/ Hypothesis**. This pilot study was designed to find the best way to characterize breast density for the study of its environmental and genetic determinants. Objectives of the study were 1) to determine whether the classification of participants into density categories using information coded from the mammography report is as precise as that using computer-aided mammographic density estimation; 2) to test agreement between density readings from two radiologists. **Methods:** 50 mammograms were selected from participants of a community medical surveillance program eligible for the main study, from three groups of women (as defined by mammography codes): cases (N=24, history of high density mammogram), controls (N=6, no history of high density mammogram), and women with intermediate density (N=20). Inclusion criteria were that the woman was 40-85 years old; had BMI ≤30 at the time of the mammogram of interest; and never had breast cancer. Mammograms were selected using stratified random sampling on calendar year of mammogram and degree of density. Mediolateral oblique views were examined by the second radiologist and then digitized using a laser film scanner. The Kappa statistic [κ] was used to assess agreement between first and second radiologist readings. Data from the scanned image was used to calculate percent density, and the resulting categorization of density was compared to the assignment based on codes.
Results: Substantial agreement was observed between density categories as determined from two radiologists' readings (weighted Kappa statistic [wK] 0.73). Perfect agreement was noted between two radiologists for high vs. intermediate and high vs. low density combinations (K 0.83 and 1.00, respectively). When grouped according to calendar year of mammogram, substantial agreement was observed for all year intervals (wK= 0.77, 0.69, and 0.70 for ≤1995, 1996-2000, >2000, respectively). Moderate agreement was observed when codes from the second radiologist reading were used to determine the density category, compared to the original density category assignment (wK 0.55). Statistical analysis of digital image data currently is underway.
Conclusions: Preliminary results showed substantial to perfect agreement between density categories, assigned based on radiologist reading. Analysis of the digital image data will direct the choice of method for the main study.

Mammary Adipose Participates in Vitamin D_3 Receptor–Induced Mammary Growth Inhibition and Tissue Maintenance

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Background: The biologically active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25D_3), is a nutritionally modulated hormone that signals through the vitamin D_3 receptor (VDR) to regulate growth and differentiation of various tissues in the body, including the mammary gland. VDR is expressed in over 80% of all breast carcinomas and *in vitro* studies have shown that 1,25D_3 induces growth inhibition, differentiation, and apoptosis of breast cancer cells. Clinical studies suggest that low serum 1,25D_3 correlates with increased breast cancer risk and metastasis. However, the mechanism and VDR-specific tissue compartment utilized during vitamin D_3-induced growth regulation and inhibition of mammary gland transformation has not yet been determined.
Objective: To distinguish the cell compartment (stromal vs. epithelial) within the mammary gland by which vitamin D_3 signals to modulate cell growth and differentiation. Our central hypothesis is that mammary adipose tissue participates as a vital endocrine component of vitamin D_3-induced negative growth regulation of normal and transformed mammary epithelial cells.
Methods: To gain additional insight into VDR signaling, VDR knockout (KO) and wild type (WT) glands were utilized for various procedures, including gene and protein expression assays, immunohistochemistry, and whole mounts, to assess the effect of VDR ablation on the aging mammary gland. Additionally, primary mammary adipocytes were cultured in the presence of vitamin D_3 to establish their impact on vitamin D_3 metabolism and signaling.
Results: Our data indicate that VDR ablation causes pathologic changes in aging mammary glands, including distension and dilation of ducts and atrophy of the fat pad. The abnormal morphological appearance of the VDR KO glands is associated with differences in gene expression, protein expression, and histological variations. Additionally, *in vitro* assays suggest that the adipocytes mediate vitamin D_3-induced growth inhibition.
Conclusions: Our work provides evidence that mammary adipose tissue contributes to Vitamin D_3-induced growth regulation in the mammary gland and represents a vital endocrine mediator of Vitamin D_3 signaling. Our data demonstrates the importance of understanding Vitamin D_3 crosstalk signaling between stromal and epithelial cells throughout mammary development, thus offering a possible mechanism by which Vitamin D_3 signals as a tumor suppressor gene.