

Poster Abstracts

Progesterone Stimulation of the Pubertal Mouse Mammary Gland

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The hormone progesterone (P) is critical for proliferation in the mammary gland during pregnancy leading to development of alveoli. However, the potential role of P during ductal development in the pubertal mammary gland is not well understood. Studies in mice and humans suggest that P influences breast cancer risk and puberty is an important period of breast cancer susceptibility, so it is essential that the role and function of P in the pubertal mammary gland be better understood. Thus, in the present study we investigated hormonal stimulation of pubertal BALB/c mice by low dose of hormones. Pubertal (3-week-old) ovariectomized mice were treated with a single dose of vehicle control (C), estrogen (E), or P and the mammary glands were analyzed 48 hours after treatment. Control treated mice showed regressed end buds and little stimulation. Mice treated with E had increased stimulation of end buds, the site of ductal elongation in the pubertal mammary gland, and increased proliferation in end buds. Notably, P alone also stimulated end buds and increased proliferation in end buds. An analysis of amphiregulin, an important mediator of E-induced end bud formation, confirmed that amphiregulin expression was increased by E, but interestingly, amphiregulin was also increased by P. Colocalization of amphiregulin with PRA, the predominant PR isoform expressed in the pubertal mouse mammary gland, showed that amphiregulin was expressed in PRA positive cells. These results suggest that P may modulate hormone-responsive pathways in pubertal mammary gland development that have been previously defined as only E-responsive. Therefore, environmental factors influencing either E or P responses in the pubertal mammary gland may affect breast cancer risk and future studies into the role of P in the pubertal mammary gland are warranted.

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Young breast cancer survivors' use of the internet to supplement oncology team regarding fertility support and informational needs

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Background: Over 240,000 women younger than 50 and diagnosed with breast cancer live in the United States and about 42,300 new cases are reported every year within this age group. During their breast cancer experience, women deal with an array of emotional and informational needs related to diagnosis, treatment, and survivorship. In particular, young women or women planning on becoming mothers seek specific information and support about the effects of breast cancer treatment on fertility.

Objectives: This study examines the perceived support and information that young breast cancer patients receive from their oncology teams, as well as patients' internet use for finding information and support regarding breast cancer and fertility.

Methods: Our research team implemented an on-line psycho-educational intervention study for young breast cancer survivors that aimed to 1) provide information and support on fertility issues resulting from breast cancer treatment; 2) examine participants' perceptions in terms of received fertility-related information and support; and 3) determine whether the internet could be used to deliver such support and information. As part the study, participants responded questions about their internet usage and their experiences with their oncology teams. Over 100 young breast cancer survivors from around the world participated in this program.

Results: Of the 82 participants residing in the United States, 9% (95%C.I.: 3.5, 16.8) expressed receiving sufficient information about fertility options from their oncology team, 22% (95% C.I.: 13.7, 32.8) received sufficient emotional support from their oncology team, and 22% (95% C.I.: 13.6, 32.5) were referred to a reproductive endocrinologist. In terms of the participants' internet usage patterns, 90% (95% C.I.:81.7, 95.7) reported being online frequently, with more hours a day spent on cancer than on fertility issues ($p<0.01$), and 87% (95%C.I.: 77.3, 93.1) participated in cancer or fertility forums. Participants reported some difficulty in finding needed information on both fertility and breast cancer issues, and relatively more difficulty on fertility issues than on breast cancer issues ($p<0.01$).

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Conclusions: These results suggest that additional tangible information and support about fertility concerns are needed by young breast cancer survivors than what they may receive from their oncology team.

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Successful Retention Strategies in Trans-disciplinary Research: the CYGNET Study

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Background: Little has been published on successful retention, compliance and dissemination strategies in longitudinal environmental exposure research involving children, or on the costs associated with such studies. Less is available regarding potential factors affecting participation and retention. The CYGNET (Cohort Study of Young Girls' Nutrition, Environment and Transitions) Study is a prospective study of 444 girls, ages 6 to 7 years at baseline exam (Year 1), investigating environmental, lifestyle, and genetic predictors of early puberty. The study includes a Community Outreach and Translation Core (COTC) that is focused on representing concerns of the advocate community and providing a channel for omni-directional communications among scientists, study participants and the community.

Objectives: The CYGNET study provides an opportunity to examine retention, compliance and dissemination strategies in longitudinal environmental exposure research involving children, as well as to examine the costs associated with these strategies – information crucial for investigators in building realistic budgets aimed at maintaining high retention, i.e., any level of commitment, which includes full participation, partial participation, or skip for a given follow-up year, and compliance rates such that the cohort remains representative of the population under study.

Methods: Recruitment began in June 2005. Baseline exams were conducted through August 2006, establishing a cohort of 444 girls. Data were collected and categorized: completion of questionnaires; collection of bio-specimens (blood, urine, buccal/saliva); anthropometry (height, weight); diet recall; Tanner staging; and psychosocial

measures. Several dissemination methods were implemented to foster continued participation: bi-annual “Tea Talk” events were created – innovative forums which bring together investigators, study staff, participants and their families to discuss the CYGNET study and related topics; newsletters; issuance of t-shirts; pedometers; gift cards; books; waiting room activities including one-on-one time with clinic staff members; reduction in visit wait times, etc.

Results: Retention rates vary over the last 3 years, depending on data collection category:

Strategies	Year 1	Year 2	Retention Rate	Year 3	Retention Rate
Cohort	444	414	93%	401	90%
Tanner staging	441	407	92%	387	88%
Questionnaire	444	414	93%	401	90%
Urine sample	422	405	96%	394	93%
Blood sample	227	286	126%	15	7%
Saliva sample	209	0	na	0	na
			% change		% change
Total Costs - % chg	\$20,029	\$26,590	32.7%	\$10,610	(60.0%)
Cost per partic. (based on Tanner staging partic)	\$45.42	\$65.33	43.8%	\$28.44	(56.5%)

(Retention rate = 1-rate of change)

Conclusions: Overall participation in the CYGNET study at the 3-year mark is remarkably stable – in excess of 93%. Whether participation can be ascribed to individual retention strategies is not clear, and suggest additional questions such as associations between cost of retention strategies and retention. Analyses will also determine whether participation in optional “Tea Talks” are associated with retention.

Pathway through puberty is associated with estradiol to estrone ratio, but not family history of breast cancer

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Background: Previous research has suggested that girls enter puberty through two pathways: pubic hair development, or breast development. Girls with breast development prior to pubic hair development have been shown to have earlier age of menarche, greater adiposity, and greater bone mineral density, factors associated with breast cancer in epidemiologic studies.

Objectives: Examine the relationship between pathway and family history of breast cancer, and between pathway and ovarian and adrenal hormones.

Methodology: Girls were seen every six months at the Cincinnati site of the BCERC. "Pathway" was defined if a girl was noted to have either breast or pubic hair development prior to development of the other manifestation of puberty. Family history of breast cancer was elicited at time of recruitment from parent/ guardian. Serum for hormone levels was obtained at every visit.

Results: When examining girls who had experienced onset of puberty, pathway could be determined in 201/237 (84.8%). Among who had pathway determined, 143/201 (71.1%) had breast development before pubic hair development. Among all participants, 11 had a mother, an additional 38 had a grandmother, an additional 3 had an aunt with breast cancer, and an additional 40 had a more distal relative with breast cancer. When examining pathway by family history, among those with a mother, grandmother, or aunt with breast cancer, 68.9% had breast development first, contrasted to 90/121 (74.4%) with no family history, ($p = .50$). When examining mean levels of serum hormones at onset of puberty, including estradiol (E2), estrone (E1), DHEA-S, and E2:E1 ratio, participants with breast development first had a greater E2:E1 ratio (.814 v .587, $p = .06$), and lower estrone ($p = .04$); all other hormone comparisons, including analyses incorporating family history, were not significantly different.

Conclusions: Pathway was not associated with family history of breast cancer, but tended to have greater E2:E1 ratio. This suggests that pathway is associated with aromatase activity, the enzyme that converts estrone to estradiol.

Pubertal regression in girls: reality or anomaly?

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Background: It is assumed puberty is a monotonically increasing scale; that is, pubertal maturation can be stable or can progress, but not regress. A previous study suggests that one gains useful information when incorporating data from girls who appear to undergo regression.

Objective: To examine the potential impact of regression in pubertal maturation for research participants in a longitudinal project.

Methods: Girls from the Cincinnati site of the BCERC were examined every six months; examiners were unaware of previous maturation status. Onset of pubertal maturation was defined by Tanner breast stages. Maturation regression was defined by pre-set rules: two consecutive visits at breast stage 1 (B1) after having the previous visit with breast stage 2 (B2); for example, (B1), B2, B1, B1, then B1 or B2. Height velocity was established by consecutive visits (6 months apart) and calculated as changes over 12 months. Age was established at the time of the visit. Serum estradiol levels were obtained at each visit, although not all have been analyzed. Comparisons were made in those without apparent regression to those who appeared to have regression. In those with regression, comparisons were made with unedited, as well as edited data.

Results: Regression of pubertal maturation appeared to occur in 42/185 (22.7%) participants. Utilizing the preset rules, age of onset of puberty was changed in 11.7% of participants, increasing the overall mean age of onset by 0.18 years. When examining impact of regression, those without regression had a mean height velocity of 6.36 cm/yr; those with regression, before editing 5.37 cm/yr ($p = .0004$), and after editing 6.04 cm/yr (not significantly different from those without regression). In those with regression, mean serum estradiol levels at time of pubertal onset were 0.179 prior to, and 0.242 after editing. However, among those with apparent regression who had estradiol levels analyzed, 2/10 participants had pubertal levels of estradiol at the "pubertal" visit which subsequently regressed to B1; estradiol levels fell back to prepubertal levels at the time of apparent regression.

Conclusions: Although height velocity data had a better fit after resolving apparent pubertal regression, some girls with regression had pubertal estradiol levels, suggesting true "physiologic" pubertal regression, perhaps related to environmental influences, such as exposure to xenoestrogens.

Mechanisms of BRMS1 Modulation of Gap Junction Inter-Cellular Communication

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The most lethal aspect of cancer is the ability of cells from a primary tumor to metastasize to other regions within the body. Breast Cancer Metastasis Suppressor 1 (BRMS1) is a metastasis suppressor gene capable of specifically inhibiting tumor cells to metastasize to secondary sites while having little or no effect on primary tumor growth. The precise mechanism of BRMS1 inhibition of metastasis is unclear, however BRMS1 associates with histone deacetylase complexes (HDACs) to modify gene transcription. BRMS1 regulates the expression of connexins which form pores in the plasma membrane and allows for inter-cellular transport of small molecules. This type of transport is known as gap junction inter-cellular communication (GJIC). GJIC is progressively lost with increasing tumor aggressiveness. Re-expression of BRMS1 in metastatic breast cancer cells inhibits metastasis and restores GJIC. The aim of this project was to determine the specific signaling mechanisms by which BRMS1 affects GJIC. Previous data have shown that BRMS1 abrogates signaling in response to TNF-alpha by decreasing NFkB activity by directly deacetylating p65/Rel A. We show here that treatment of metastatic MDA-MB-435 cells with TNF-alpha decreases connexin 32 protein levels whereas TNF-alpha treatment has no effect on connexin expression in MDA-MB-435BRMS1 cells. We propose a mechanism whereby BRMS1 may affect GJIC through modulation of connexin expression via NFkB.

The Angel Squad

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Background: The mission of the Angel Squad is to provide comfort, hope and compassionate services to breast cancer patients and to their caregivers at the University of Alabama at Birmingham.

Methods/Work Performed: To provide emotional and psychological support to breast cancer patients, caregivers and clinic staff. To advocate for the patient in a complex academic medical environment. To identify patients with special needs, and help them access resources to meet those needs. To plan, implement and evaluate educational programs and materials for patients and their families. To encourage participation in clinical trials.

Evaluation/Outcomes: Patient satisfaction with service and care has improved and Angel volunteers are cited as having made a difference. The majority of the patients evaluated in The Interdisciplinary Breast Cancer Clinic are self referrals who heard of the Clinic from a previous patient. Over 90% of patients seeking a second opinion choose to have care within the UAB system. Many cite the compassion and care provided by staff and volunteers as vital to their choice.

The Angel Squad is the only volunteer organization within The Kirklin Clinic, and it now serves as a model for other programs being developed within the Clinic and in the Comprehensive Cancer Center.

Angels support each other and say they are very proud and satisfied with their volunteer role.

Family Viewed: The Survey Says...

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Background: The Community Outreach and Translation Core (COTC) is a project within the Breast Cancer and the Environment Research Centers (BCERC). One objective of the COTC is to communicate/translate study findings to the general public, study families and breast cancer advocates. The Cincinnati COTC has done this by designing educational materials and conducting programs for these audiences.

With any longitudinal study, retention of study participants can be a challenge. Appropriate communication between researchers and participants can be an important factor in the success of multi-year studies. The families of the girls who participate in the Growing Up Female study (Project 2 of the Cincinnati BCERC) have not been systematically surveyed regarding their preferences for communications about the study and/or related topics. No data has been collected to assess their interest in receiving study findings and/or other information.

Objectives: A self-administered questionnaire was developed to determine if the study families want more information related to study specific results or general health information on topics related to the study. They were also asked how they would prefer to receive the information. Data collected *via* the survey will be used to design communications for the families related to the information they desire, using methods that are most responsive to their expressed preferences.

Methods: The Cincinnati BCERC COTC, with input from Project 2, developed a questionnaire for parents/guardians of girls participating in the Growing Up Female study. The anonymous surveys were mailed to each participating family in August 2008 and included a self-addressed, stamped envelope for return. Survey questions probed respondents' interest in receiving information for them or for their child related to 1) their child's study results, 2) aggregate overall study results, and/or 3) general health topics related to the study. Preferred methods for receiving the information were solicited as well. Study families' overall satisfaction/dissatisfaction with study logistics was also explored with opportunities to give suggestions for improvement. Returned surveys were assigned a unique identification number and responses were entered in an electronic database. Data are being analyzed using SPSS.

Results: The survey responses will be presented and summarized. Preliminary plans to respond to recommendations for improving study logistics will be discussed. Goals and strategies to communicate/translate study findings and identify age-relevant health related information consistent with the survey findings will be outlined.

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Father Absence and Girls' Puberty in the Bay Area Breast Cancer and the Environment Research Center (BABCERC)

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Background: Early life events play an important role in the development of breast cancer, and girls' pubertal timing is thought to be a key event. The link between menarche and breast cancer is of particular concern, given that the average age of pubertal onset among U.S. females is declining. In addition to hereditary factors, environmental and behavioral factors influence timing of puberty. The most commonly suggested "lifestyle" determinant is obesity; however, girls' BMI only partially explains the secular trend in pubertal timing. Lesser known, but also important, determinants are characteristics of the family environment. Longitudinal and national probability studies have established that family factors – particularly biologic father absence – significantly affect puberty. Analogous animal studies confirm the important role of family composition and environment.

Objective: To examine the effect of father absence at 6-8 years old, on psychological outcomes, family environment, BMI and pubertal status one year later.

Method: In the epidemiologic study of the BABCERC (i.e., the CYGNET study), we have assessed child and maternal psychopathology, family environment, and family composition, breast development and other anthropometric factors (e.g., height, weight). Participants, who were recruited from Kaiser Permanente Northern California, are 444 6-8 year-old girls (26% Latina, 21%

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African-American, 42% non-Latina White, 11% Asian) and their caregivers (primarily mothers). Caregivers completed measures of family and psychological functioning and family composition. Preliminary analyses include calculation of descriptive statistics for study variables. Regression analyses will be conducted to determine if baseline father absence predicts maternal/child psychological problems, familial dysfunction, BMI and pubertal status (breast development) one year later.

Results: Descriptive statistics will be presented. At baseline, 70% of girls lived with both a biologically-related mother and father, which is consistent with 2007 U.S. Census data indicating 72% of 6-8-year-old children live with both biological parents. Only 3% of girls had non-related adult males in their homes, precluding analyses with non-related males. Preliminary baseline analyses suggest that girls living in father absent homes exhibit higher BMI but not accelerated puberty. This was expected given the young age of the girls and limited number of transitions to Stage 2 of breast development. Results from regression analyses predicting Y02 outcomes, including pubertal status, will be presented.

Implications: By seeking to identify modifiable mechanisms involved in pubertal timing, including family functioning and girls' overweight, the current research has potentially significant clinical implications for girls' health in the short term and for breast cancer later in life.

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***In utero* resveratrol and 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin treatments alter mammary gland development.**
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2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (TCDD) is a dioxin produced during the manufacture of herbicides and germicides and during the incineration of municipal and industrial waste. It has been shown to cause urogenital deformities and alteration of mammary gland structure. Preliminary data shows that it also increases tumorigenesis. Resveratrol is a compound found in grapes and grape products and is known to have antioxidant and anti-inflammatory effects. It has also been shown to reduce mammary tumorigenesis, incidence, and multiplicity in a chemically-induced rat model. This study investigates the potential of resveratrol and TCDD delivered *in utero* to alter the development of mammary glands. The hypothesis is that *in utero* TCDD treated mammary glands will be predisposed to tumorigenesis by developing increased numbers of terminal end buds (TEBs), highly proliferating structures associated with breast cancer, and that resveratrol treated mammary glands will be protected from tumorigenesis by a decreased number of TEBs. Pregnant rats were placed on a diet of either AIN-93A or 333 mg resveratrol/kg AIN-93A diet throughout the pregnancy. On gestational day 15, animals were gavaged with either 3 µg TCDD/kg BW or an equivalent amount of sesame oil (SO). In this way, four treatment groups were created: AIN-93A/SO (93A/SO), resveratrol/SO (Resv/SO), AIN-93A/TCDD (93A/TCDD), and resveratrol/TCDD (Resv/TCDD). At birth, offspring were transferred to a surrogate mother fed AIN-93A throughout pregnancy.

Results showed that mammary glands of 50 day old rats from the resveratrol treated groups were significantly larger in square area than control and 93A/TCDD treated glands. This result is not an effect of animal weight. 93A/TCDD treated glands were found to have more total ductal structures per mm² than all the other groups. This includes a significantly higher number of TEBs than all the other groups. 93A/TCDD also had a significantly higher number of lobules, mature, low proliferating structures, than the control group. Resveratrol did not affect the mammary gland morphology. For all ductal structures, there was no significant difference between the resveratrol treated groups and the controls. Therefore, our hypothesis is partially correct. 93A/TCDD treated animals have larger numbers of ductal structures, including TEBs which could predispose the mammary gland to cancer. However, the potential protective effects of resveratrol do not appear to be linked with mammary gland morphology. (Supported by NIH 1R21ES015603-01)

BRMS1 suppresses breast cancer metastasis, in part, by regulating microRNA expression

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Metastasis is the major cause of morbidity and mortality in cancer patients. Breast cancer Metastasis Suppressor 1 (BRMS1) suppresses metastasis of multiple carcinomas without blocking tumorigenesis. Functionally, BRMS1 interacts with SIN3:histone deacetylase complexes and modifies gene expression. We recently discovered that a subset of the BRMS1 regulated transcriptome included microRNA. BRMS1 was also found to down regulate the metastasis-promoting miRs 373, and 520c and increase the metastasis suppressing miRs 146a/b and 335 in MDA-MB-231 and 435 cells. Given these data, we hypothesized that BRMS1 suppresses metastasis through regulating miRNA. Given that miR-146a/b regulate NFκB, a known target of BRMS1, and that miR146a/b show the greatest change with BRMS1 re-expression (6-60 fold), these miRNA were chosen to evaluate as potential mediators of BRMS1 metastasis suppression. When transduced into metastatic MDA-MB-231 cells, miR-146a/b suppressed experimental metastasis by 69 and 84% respectively when compared to vector control cells in a xenograft mouse model system (mean pulmonary metastases: vector control = 39 ± 6, miR-146a = 12 ± 1, miR-146b = 6 ± 1). This would indicate a role for miRs 146a/b in BRMS1 metastasis suppression. These data suggest that regulation of metastasis associated miRNA by BRMS1 is an important part of how BRMS1 suppresses metastasis. Moreover these data provide support for the notion that modulating levels of miR genes, in particular miRs 146a/b, could have therapeutic potential for breast cancer metastasis.

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Chemoprevention of Mammary Carcinogenesis by Dietary Methylselenocysteine: Effects on Circadian Rhythm and Estrogen Receptor β Cycling

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The chemopreventive efficacy of the naturally occurring amino acid, methylselenocysteine (MSC), was first revealed by Ip and coworkers who demonstrated that garlic grown in selenium enriched soil could reduce the incidence of chemically-induced rat mammary tumors by ~60%. Epidemiological studies further demonstrated that organic selenium supplements reduced the incidence of several cancer types in geographical areas with low soil selenium levels. To further define the molecular basis for MSC mediated chemoprevention, we compared global gene expression profiles in normal mammary tissues from pubescent female rats maintained on a diet supplemented with MSC (3 ppm Se), or a standardized AIN-76A diet (0.1 ppm Se), with and without exposure to carcinogen. The results of this analysis indicated that a single, carcinogenic dose of NMU ablated, and that dietary MSC supplementation restored, the coordinated diurnal expression of circadian rhythm genes in mammary epithelial cells. Moreover, MSC-induced expression of circadian genes was lost in all tumors that arose in the ~30% of NMU treated animals maintained on the MSC enriched diet. Together, these findings suggested that a normal circadian rhythm suppressed mammary carcinogenesis. We further demonstrated that neither carcinogen exposure nor dietary MSC altered blood melatonin levels, indicating that neither treatment had an effect on light mediated regulation of melatonin through the Suprachiasmatic Nucleus. By contrast, MSC induced a dramatic increase in the expression of the melatonin receptor in mammary cells, suggesting that increased melatonin signaling contributed to the MSC-mediated restoration of circadian rhythm in NMU treated mammary cells. More importantly, we demonstrated the MSC induced a 7-fold increase in the circadian expression Estrogen Receptorβ (ERβ) in the mammary tissue of carcinogen treated rats. These findings suggested that dietary MSC prevents mammary carcinogenesis through ERβ-mediated differentiation and growth inhibition in mammary epithelial cells. Significantly, epidemiological studies showed that occupational disruption of circadian rhythm is also associated with an increased risk of breast cancer. Future research will therefore test the hypothesis that abnormal cycling of ERβ, resulting from disruption of circadian rhythm, contributes to breast cancer in shift workers.

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Environmental factors and breast cancer risk: analysis of organochlorine and polychlorinated biphenyls in human breast tissue and serum.

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Background: Exposure to environmental pollutants is likely a major risk factor for the development of breast cancer. Prior studies analyzing serum levels of organochlorines and polychlorinated biphenyls (PCBs) have not demonstrated elevated levels in breast cancer patients. This IRB approved study was undertaken to compare levels of the substances in serum and tissue for patients with breast cancer and benign breast disease. The study participants were located in the Hudson river valley in New York. This region has a large agricultural base and elevated PCB levels have been identified in the Hudson river.

Objective: To assess the impact of organochlorines and PCBs on the risk of developing breast cancer.

Methods: Study design involved a nonrandomized, nested case-control of 101 women undergoing breast surgery. 75 patients with breast cancer and 26 patients with benign disease also completed a comprehensive questionnaire focusing on lifestyle, dietary and environmental factors. 15 ml of Serum and 1 gram of fatty breast from each patients were collected for analysis of a panel of 15 organochlorine pesticides and 30 PCB congeners. This analysis was performed using mass spectroscopy by the department of environmental health and toxicology in Albany, NY. Spearman correlation coefficient and non-parametric Wilcoxon rank test were used to analyze the data. Funding for the project was provided by the Benedictine Health Foundation, Kingston, New York.

Results: Analysis of the correlation data between serum and tissue showed no correlation for oxychlorodane and mirex ($r=0.12$, $p=0.25$) while a low correlation ($r=0.23$, $p=0.025$) for DDT was found. The other compounds had medium or high correlation. This suggests that serum levels may not accurately reflect tissue levels for certain environmental substances. Comparison of levels for breast cancer patients versus patients with benign disease showed significantly higher levels of tissue PCB congeners IUPAC 118,138,153,180 and DDE ($p=0.049$) in breast cancer patients. Serum levels of IUPAC 153, HCH and DDE were higher in breast cancer patients ($p=0.08$).

Conclusion: This study suggests that chlorinated pesticides and PCBs may have a role in breast

carcinogenesis. Serum levels may not accurately reflect the breast tissue exposure. A larger breast tissue multi-center study with patients more diverse environmental exposures would be helpful.

Bisphenol A (BPA) induces neoplastic transformation in human breast epithelial cells in vitro.

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Bisphenol A (BPA) is an endocrine disruptor because of their capacity to perturb normal hormonal actions by competitively binding and activating endogenous estrogen receptor (ERs), with an affinity 10,000-fold less than that of E₂ for both ER α and ER β . In this work we aim to determine that BPA is carcinogenic in human breast epithelial cells (HBEC). We have used for this purpose MCF-10F, a spontaneous immortalized HBEC, that is ER α and Progesterone receptor negative and weakly positive for ER β . We treated the cells with 10⁻⁶M, 10⁻⁵M, 10⁻⁴M and 10⁻³M BPA during two weeks adding fresh media every day, using DMSO (vehicle) as control. The 10⁻³M and 10⁻⁴M BPA were toxic. BPA at the concentration of 10⁻⁶M, and 10⁻⁵M induces a significant lost of the ductulogenic and branching capabilities in collagen matrix with the concomitant formation of solid masses (>100 μ m in diameter)(27 and 20%, respectively). These masses were lined by a multilayered epithelium with alteration in the cell polarity. BPA transformed cells were highly invasive using the Boyden chamber. The invasive cells that passed through the membrane were recovered, expanded and injected in SCID mice to test the capacity of these cells to produce tumors. In conclusion, we demonstrate for the first time that the BPA treatment of the human breast epithelial cells MCF-10F induced the lost of the branching on collagen matrix with the concomitant formation of solid masses indicating that BPA induce cell transformation and invasiveness in Matrigel. BPA at 10⁻⁵M showed to be acting with the same potency and efficiency than the natural estrogen (17- β -estradiol) by changing the normal phenotype of the cells on collagen and acting as a complete carcinogen. (This work was supported by grants NCI and NIEHS Grant UO1 ES012771 and R21-ES15894)

Characterization of Antisense Transcripts in the Mouse Progesterone Receptor Gene

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Experiments using quantitative PCR (Q-PCR) targeted to various regions of the mouse progesterone receptor (PR) gene showed discordant results comparing mammary cell lines known to differ with respect to their level of PR expression. Based on *in silico* evidence from GenBank and *in vitro* evidence from an analysis of mouse tissues and cell lines, the PR locus appears to contain a poorly defined antisense promoter at the 3' end of PR exon 1 that gives rise to antisense transcripts spanning exon 1. These antisense transcripts interfere with the ability of the Q-PCR assay to measure sense PR message using cDNA templates primed with oligo(dT) or random hexamer primers. Similarly, a recent report presents evidence for antisense transcription within the PR locus of two human tumor cells lines [Schwartz JC, *et al.*, 2008]. These antisense transcripts initiate within PR exon 1 in both the mouse and the human PR gene. The antisense transcripts are polyadenylated, non-coding RNAs (ncRNAs) that are unspliced in mouse, but can be either spliced or unspliced in the human. In both species, the PR gene and its antisense transcripts form sense/antisense pairs showing a divergent orientation (5' overlap) with the antisense transcript overlapping the majority of sense PR exon 1, including a significant portion of the PR protein reading frame. *In silico* evidence to support the existence of a PR antisense transcription unit in the mouse consists of: cap analysis of gene expression (CAGE) tags, expressed sequence tag (EST) clones, and Riken cDNAs. This is supported by *in vitro* evidence of antisense transcription in the mouse PR locus, including: primer extension analysis, strand-specific semi-quantitative PCR, transient transfection experiments using an antisense reporter construct, and *in situ* hybridization. In human mammary gland cell lines, the antisense transcript was reported to interact *in vivo* with chromatinized PRB promoter DNA using co-immunoprecipitation techniques and therefore may contribute to differential regulation of transcription of the PRB isoform. It is proposed that a similar interaction may also occur in the mouse PR locus, with antisense transcripts affecting the balance between PRB and PRA transcription in reproductive tissues such as the mammary gland.

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State of the Evidence: The Connection between Breast Cancer and the Environment

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In March 2008, Breast Cancer Fund (BCF) released the 5th edition of our landmark report, *State of the Evidence: The Connection between Breast Cancer and the Environment*. SOE 2008 advances the scientific conversation by aggregating the evidence linking toxic exposures and breast cancer. In this edition, BCF made several key changes to content and format. The report is split into three sections: Framework, Evidence and Moving Forward. The Framework section reviews the larger, overarching issues that have emerged in the past two years of research including the impact of early life exposures, low dose exposures and cumulative and synergistic effects of exposures on breast cancer risk. In conceptualizing the contributions of the many factors involved in enhanced risk for breast cancer, we have posited a model that acknowledges the web-like complexity of interactions between environmental factors and genetic, lifestyle, reproductive, age-related and other risk factors for the disease. The Evidence section summarizes the science to date but instead of outlining in terms of known, probable and possible environmental links to breast cancer as in past editions, we chose to organize based on more logical families of chemical and radiation exposures while still including the National Toxicology Program (NTP) and International Agency for Research on Cancer (IARC) designations of carcinogenicity. The Moving Forward section includes BCF's recommendations for research and state and federal policy that, once implemented, will reduce the public's exposure to toxic chemicals and radiation. This section, in particular, provides a substantive platform from which BCF will launch advocacy campaigns. Also new in this edition are sidebars that highlight key areas of concern for consumers like cosmetics, plastics, and pesticides and other important analyses on emerging issues like nanotechnology.

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Harnessing Action Tendency Emotions to Induce Mothers to Encourage Healthy Eating in Their Preadolescent Daughters

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Background: Research has shown that girls between the ages of 6 and 11 are influenced by their mothers' attempts to control their eating habits, and that action tendency emotions can be used successfully as persuasion techniques if the proper emotion is elicited. Emotions are defined as internal mental states that result from an evaluation of people, events, or objects and action tendency refers to the ability of an emotion to promote some type of problem solving activity. This investigation explored action tendency emotions as persuasion techniques to encourage mothers of preadolescent daughters to influence their daughters to eat healthily.

Hypothesis/Objective: RQ1: What types and sources of messages encouraging preadolescent girls to eat a healthy diet will elicit certain emotions in mothers? RQ2: Will the elicitation of any of the action tendency emotions (anger, guilt, sadness, fear, happiness, compassion, and pride) be more likely to influence mothers of preadolescent daughters to encourage healthy eating?

Method: Four focus groups (N=14) were conducted with a semi-structured protocol intended to elicit participants to report on situations involving their daughters' diets that could cause them to experience emotions of anger, guilt, sadness, fear, happiness, compassion, and pride, including the source(s) and types(s) of messages.

Results: The sources most likely to elicit certain emotions were: *anger*: mother in law, another mother; *happiness*: her own mother; *sadness*: study/report, another child, daughter; *compassion*: another child, daughter; *guilt*: another mother, daughter, herself, her own mother; *pride*: friends; and *fear*: parenting magazines, study/report, the unknown.

The responses participants predicted for certain emotions were: *anger*: constructive response if helpful information is provided, *happiness*: maintain current efforts or possibly be motivated to work harder, *sadness*: may not do anything especially if it is too late, *compassion*: engage in helping behaviors, *guilt*: change behavior if new helpful information is provided, *pride*: maintain efforts or possibly work harder, and *fear*: behavior change if it is possible to do so, especially if paired with a concrete action to be taken.

Conclusion: The information demonstrated that behavior change and/or maintenance may be achieved through certain action tendency emotions. This formative investigation suggests that sadness, compassion, guilt and fear can be elicited by the most sources, and that anger, compassion, guilt and fear are more likely to result in

behavior change, while happiness and pride are most likely to result in behavior maintenance.

Association of Age at Menarche with Adult Leg Length and Trunk Height: Speculations in Relation to Breast Cancer Risk

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Background: Given that earlier age at menarche predicts for shorter stature, it seems paradoxical that breast cancer risk is associated with early menarche, as well as with increased height. We investigated age at menarche and adult measured standing height, leg length and trunk height to further characterize this relationship.

Methods: A two-generation cohort was used; retrospective data from the parental generation and telephone interviews and in-person anthropometric measurements in their daughters performed. Multivariable linear regression analyses were conducted using mixed regression models to account for same-family participants.

Results: Controlling for birth weight, maternal height, and birth cohort, and analyzed as a group, an increase in the age at menarche of one year predicted an increase in standing height, leg length, and trunk height of 0.76 cm, 0.41 cm, and 0.35 cm, respectively. However, when stratifying by birth year (birth prior to 1966 vs 1966 or after), the relationship between menarche, height, and leg length was true only for those born prior to 1966.

Conclusions: This study demonstrates that for adult women born prior to 1966, a later age at menarche predicts for both longer leg length and increased trunk height, indicating a similar amount of growth between trunk height and leg length prior to the onset of menarche. However, for those born in 1966 or later, the relationship is no longer present.

Conclusion: Age at menarche continues to fall while the average height of girls in the US continues to rise. Given the link between height and breast cancer risk, and the finding in this study that the relationship between age at menarche and adult height no longer exists in women born in 1966 or later, it is possible that the time-honored relationship between age at menarche and breast cancer risk may also no longer exist. Further studies are necessary to explore these findings and their association with breast cancer risk in recently established cohorts.

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The second predicted nuclear localization sequence of BRMS1 is required for metastasis suppression

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Breast cancer metastasis suppressor 1 (BRMS1) is a predominantly nuclear protein that suppresses metastasis in multiple human and murine carcinoma cell lines. BRMS1 interacts with several nuclear proteins including SIN3:HDAC chromatin remodeling complexes and NFκB that are involved in repressing transcription. We hypothesized that nuclear localization of BRMS1 was essential for BRMS1-mediated breast cancer metastasis suppression. BRMS1 has two predicted nuclear localization sequences (NLS) based on PSORT II that are located near the C-terminus (amino acids 198-205 and 238-244, NLS1 and NLS2 respectively). To increase the size of BRMS1, above that freely diffusible into the nucleus (>60 kDa), GFP and GST chimeric proteins were generated. Replacement of NLS2 with NLS1 (BRMS1^{NLS1,1}), truncation at 238 (BRMS1^{ΔNLS2}), or switching the location of NLS1 and NLS2 (BRMS1^{NLS2,1}) had no effect on nuclear localization, however, replacement of NLS1 with NLS2 (BRMS1^{NLS2,2}) or truncation at 197 (BRMS1^{ΔNLS}) localized to the cytoplasm. These data demonstrate that NLS1 is necessary for nuclear localization. Metastatic breast cancer cells, MDA-MB-231, transduced with BRMS1^{NLS1,1} or BRMS1^{NLS2,2} (but not the chimeric GFP-GST) were evaluated for their ability to suppress metastasis in an experimental xenograft mouse model. Interestingly, while NLS2 was not required for nuclear localization, it was found to be important for metastasis suppression since BRMS1^{NLS2,2} but not BRMS1^{NLS1,1} suppressed metastasis. Consistent with this data, the pro-metastatic transcription factor TWIST1 was down-regulated by re-expression of BRMS1 in MDA-MB-231 cells, but not by BRMS1^{NLS1,1}. Together, these data suggest an important function of NLS2 for metastasis suppression that is distinct from nuclear localization.

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Chronic Oral Bisphenol A Exposure During Adulthood Accelerates Mammary Carcinogenesis in Mice

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Background: Bisphenol A (BPA) is used in the production of a myriad of consumer goods, such as food and beverage containers, canned food and drink liners, and some dental sealants. Studies have shown that BPA leaches from these products in appreciable amounts, resulting in upwards of 90% of the populations studied having detectable concentration of BPA metabolites. Because of BPA's estrogenic and endocrine disrupting effects, it is suspected to potentially play a role in the high incidence and progression of breast cancer.

Objective: We hypothesize that BPA is harmful to women with established breast pathology that is responsive to hormones and/or growth factors. We believe that under these circumstances BPA is capable of contributing to breast cancer development and progression.

Methods: Mice engineered to overexpress wildtype erbB2 were given approximate exposures of 0, 5, 50, and 500 μg BPA/kg BW/day from eight weeks of age until sacrifice. The selected doses represent a realistic daily exposure value (5μg BPA/kg BW/day, 5BPA), the US EPA's "safe" threshold dose (50μg BPA/kg BW/day, 50BPA) and a 100-fold reduction of the reported lowest observable effects level (500μg BPA/kg BW/day, 500BPA). As we expect the effects of BPA to be mediated through an estrogen-like mechanism, we have also included a positive control treatment of estradiol (10.8μg estradiol/kg BW/day, E2).

Results: Chronic exposure to BPA during adulthood significantly decreased tumor latency and increased multiplicity. Median time to palpable tumor was recorded as 239, 205, 223, and 203 days for control, 5BPA, 50BPA, and 500BPA. The 5BPA treatment group had a significant increase in tumor multiplicity (p=0.0097) as compared to the negative control. While body weight was significantly lower for all treatment groups (5BPA, 50BPA, 500BPA and E2), only 50BPA (p=0.01), 500BPA (p=0.009), and E2 (p=0.04) had significantly greater uterine-to-body weight ratios as compared to control mice.

Conclusions: Chronic consumption of BPA during adulthood accelerates several parameters of mammary carcinogenesis. Interestingly, it is the lowest dose of BPA which shows the most potent effects. Of special interest is the estrogenic effects noted by increased uterine to body weight ratios in 50BPA and 500BPA, mirroring the effects of chronic E2. This effect was absent from 5BPA, suggesting that tumorigenesis effects observed for 5BPA and 500BPA may have distinct molecular mechanisms of action.

Early exposure to bisphenol A imparts increased tumorigenesis and long-lasting alterations to protein expression in the mammary glands of adult rats

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Background: Bisphenol A (BPA) is widely used in the manufacture of polycarbonate plastics, including infant formula bottles. BPA has been found to leach from polycarbonate infant formula bottles and has been detected in colostrum and breast milk, suggesting several sources for early BPA exposure and creating the concern that long-lasting, adverse health effects may arise as a consequence.

Objectives: Based on the reported estrogen-like activity of this polyphenol, we hypothesized that exposure to BPA early in life would elicit developmental changes in the mammary tissue and predispose for mammary cancer.

Methods: Neonatal/prepubertal rats were exposed to biologically relevant doses of BPA *via* lactation from nursing dams treated orally with 0, 25, and 250 µg BPA/kg body weight/day. For tumorigenesis studies, female offspring were exposed to 30 mg dimethylbenzanthracene (DMBA)/kg body weight at 50 days postpartum.

Results: The combination of DMBA treatment after lactational exposure to BPA demonstrated a dose-dependent increase in mammary tumor multiplicity and reduced tumor latency compared to control. In the absence of DMBA treatment, lactational BPA exposure resulted in increased cell proliferation and decreased apoptosis at 50 days, but not 21 days (shortly after last BPA treatment), postpartum. Using western blot analysis, steroid receptor co-activators (SRCs) 1-3, Akt, phospho-Akt, progesterone receptor A (PR-A), and erbB3 proteins were determined to be significantly up-regulated at 50 days postpartum.

Conclusions: This data provides the first proof that early exposure to BPA through an oral route accelerates DMBA-induced mammary carcinogenesis. We conclude that increased cell proliferation and decreased apoptosis at the time of DMBA administration plays a vital role in BPA's mechanism of action. We believe that these effects are, at least in part, mediated through increased expression of erbB3 and the p160 family of steroid receptor co-activators (SRC-1, SRC-2, and SRC-3) and increased Akt activity. Interestingly, the published observations of early exposure to BPA overlap well with the reported phenotypes of transgenic mouse models of SRC deficiency.

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Evaluating the Impact of Adult Exposure to Bisphenol A on Women with Breast Cancer.

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Background: Bisphenol A (BPA) is used to manufacture polycarbonate plastic and epoxy resins. These plastics are used in a plethora of commonly used consumer goods, creating the potential for widespread exposure. Indeed, several studies have shown greater than 90% of those surveyed having detectable metabolites of BPA. By most accounts, BPA is thought to potentially contribute to breast cancer incidence through its weak estrogenic activity. This creates the concern that chronic exposure of BPA could "feed" breast tumors either directly, through binding to the estrogen receptor, or indirectly, through "crosstalk" with growth factors. Ongoing animal studies using mice genetically engineered to develop mammary tumors that overexpress erbB2/neu (rodent equivalent to HER2) have shown that BPA is capable of accelerating mammary tumorigenesis at concentrations that are physiologically attainable by humans.

Hypothesis: We are investigating whether BPA exposure in women with estrogen receptor and/or erbB2/HER2-positive breast cancer is correlated with altered sex steroid and growth factor signaling within the tumors.

Methods: We are actively recruiting women newly diagnosed with breast cancer through the UAB Breast Health Clinic. Paired urine (collected on the day of consent) and remnant breast tumor tissue is being collected from each woman recruited into the study. We will use the urine to measure both free (parental) BPA and the BPA metabolite, glucuronide-BPA. Western blotting on the tumors will then be used to assess expression of several proteins (phospho-erbB2, phospho-erbB3, Akt, phospho-Akt, SRC-1, SRC-2, SRC-3, IGFR, ER alpha, ER beta, PR-A, PR-B, etc.) known to play a role in tumor progression. As we expect the effects of BPA to be mediated through the estrogen receptors and erbB2/HER2 receptor in the breast tumors, we will use triple-negative breast tumors as a negative control, where BPA concentrations should not be significantly correlated with alterations in protein expression in the breast tumor. This project has been approved by the UAB IRB (F070823001).

Results: To date, we have recruited and collected for 24 women. Stratifying these women according to tumor hormone and growth factor receptor status, we have: 2 ER/PR/HER2+, 14 ER+/PR+/HER2-, 3 ER+/PR-/HER2-, and 5 ER/PR/HER2- tumor samples.

Amphiregulin: a novel paracrine mediator of progesterone-induced proliferation in the adult rat mammary gland

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The exact molecular mechanisms of how P regulates proliferation in the normal breast are largely unknown. In the breast, P produces its biological effects by acting on two major progesterone receptor isoforms, PRA and PRB. The rat mammary gland closely resembles the human breast with regard to the pattern of cellular expression of PR isoforms and provides an excellent model for the study of P action in the breast.

The objective of this study was to investigate how P acting through PRA and/or PRB induces proliferation in the adult rat mammary gland. Adult Sprague-Dawley rats were bilaterally ovariectomized and treated with vehicle control, estrogen alone (17 µg/kg), progesterone alone (15 mg/kg), or combination of E and P for 3 consecutive days. Very few proliferating cells were found in OVX control mammary glands. P alone did not induce proliferation, E alone only slightly increased proliferation, whereas E+P treatment had a strong synergistic stimulatory effect on proliferation. Immunohistochemical analysis showed that E was required to restore PRA expression after OVX, whereas PRB expression was not affected by OVX. The majority of proliferating cells expressed only PRB, about 25% of proliferating cells expressed both PR isoforms (PRA+PRB+), while PRA only expressing cells rarely proliferated.

To further investigate the mechanisms of P action, we performed experiments using 3D culture of the mammary organoids. Interestingly, in culture, P alone had a potent stimulatory effect on luminal cell proliferation. E had no effect on proliferation and was not required for the maintenance of PRA expression. Based on our observations, we hypothesized that 1) PRA expression is required for the proliferative action of P, 2) P acting via PRA induces paracrine factor(s), and 3) these paracrine factors are required for P-induced proliferation mediated by PRB.

Amphiregulin (Areg), a ligand of the epidermal growth factor receptor, may be one such paracrine factor. Indeed, E+P treatment but not E or P alone induced expression of Areg in PRA+PRB+ cells in OVX rats. In cultured mammary organoids, Areg stimulated proliferation of the luminal epithelial cells similar to P treatment. Areg may also be an important paracrine factor that mediates the proliferative action of P in the adult human breast.

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The Effects of the Framing of Memorable Breast Cancer Messages on Leading People to Engage in Detection or Prevention Behaviors

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Background: Memorable 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" (p. 27). They have also been shown to be guides to actions such as practicing health behaviors. Message framing, which refers to emphasizing either the benefits (gain-framed) or the costs (loss-framed) of a behavior, suggests that gain framed messages are ideal for promoting disease prevention behaviors, whereas loss-framed messages are better for promoting detection behaviors. Previous research usually creates framed messages to experimentally determine their effects. Few studies have examined a set of self-reported messages about a particular health issue such as breast cancer to determine if they are gain or loss framed and to assess their effects on health behaviors.

Methods: Three hundred fifty-nine women were recruited to take an online survey regarding meaningful messages they recalled about breast cancer. A total of 217 women recalled such a message, described it, and noted whether it had resulted in prevention (healthy diet and exercise) or detection behaviors (mammograms and breast self-exams).

Hypotheses/Objectives: In accordance with previous research, the hypotheses are that gain-framed messages will be associated with breast cancer prevention behaviors while loss-framed messages should be associated with detection behaviors. To test this, we will determine the framing of the self-reported messages by examining the "kernel states" mentioned in the message, such as a healthy life, breast cancer, or death. Whether or not these kernel states are desirable or undesirable, or avoided or attained, will tell us which type of framing they embody:

	Undesirable kernel state	Desirable kernel state
Avoided kernel state	Gain-framed	Loss-framed
Attained kernel state	Loss-framed	Gain-framed

Thus, we pose the following research questions and hypotheses:

- RQ1. What percentage of messages are framed?
- RQ2. What percentage of messages are gain-framed due to an undesirable kernel state that is avoided?

- RQ3. What percentage of messages are gain-framed due to a desirable kernel state that is attained?
- RQ4. What percentage of messages are loss-framed due to an undesirable kernel state that is attained?
- RQ5. What percentage of messages are loss-framed due to a desirable kernel state that is avoided?
- H1: Gain-framed messages will be significantly associated with prevention behaviors.
- H2: Loss-framed messages will be significantly associated with detection behaviors.

Results & Conclusions: Preliminary analyses have shown that 23.1% of the messages reported are framed. Of those that are framed, 81.25% are gain-framed, and 18.75% are loss-framed. The majority of the messages (75%) contain desirable kernel states, and the most common form of the framed messages is a desirable kernel state that is attained (68.75%). Additional results are forthcoming and will be included on the poster at presentation time.

The effect of pre-pubertal exposure of Benzyl Butyl Phthalate (BBP) on the rat mammary gland.

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Benzyl Butyl Phthalate (BBP) is an identified carcinogen that has been linked to breast cancer through studies conducted on Sprague Dawley rats. BBP is a commonly used plasticizer found in toys, PVC, cosmetics, and carpeting. This phthalate is absorbed into the body through inhalation, dermal and oral exposure and accumulates in the fatty tissues of the body. BBP can also be passed from mother to offspring through the placenta and during lactation. BBP is capable of binding to estrogen hormone receptors. The purpose of this study was to examine the effects of BBP on the mammary glands of Sprague Dawley rats. The animals were exposed to BBP through mother's lactation at a concentration of 500µg/kg of body weight or equivalent volume of sesame oil (control group). At 50 days of age the animals were sacrificed and the mammary gland submitted to morphological study through whole mount preparation. The number of terminal end buds (TEBs) was counted in the abdominal mammary glands for both groups (exposed to BBP and control). The effects of the BBP were further analyzed by using cDNA-microarrays to compare the gene expression profile between the experimental and the control rats. There were not morphological differences between the control and experimental group. However, there were 80 genes significantly different in the mammary gland of BBP exposed animals compared with the matching control. The genes differentially expressed are involved in controlling the circadian rhythm such as Dopa decarboxylase (Ddc), organ development, androgen and estrogen receptors and apoptosis. These genes potentially play a role in the development of cancer cells. In conclusion, pre-pubertal exposure to BBP did not alter the mammary gland structure, but it modifies their genomic profile.

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Approaches to RNA extraction from whole blood, saliva, and urine in prepubertal girls

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Background: Xenobiotics known as endocrine disruptors (EDCs) can have estrogenic activities through multiple mechanisms, such as interacting with estrogen hormone receptors or influencing the synthesis or metabolism of endogenous hormones. The molecular mechanisms through which EDCs act have not been well defined, but it is assumed that EDCs act through transcription and/or translation of genes, leading to production of RNA and ultimately protein products.

Objectives: To examine the optimal methods for RNA extraction from saliva, whole blood and urine in a group of girls that can provide the best RNA quality for performing genomic studies using Agilent Microarrays.

Methodology: Girls were participants of the Cincinnati site of the BCERC. Blood has been drawn every six months in EDTA tubes, with isolation of a buffy coat and preserved in RNA-later. The PAXgene system was used in a small cohort for direct blood collection. Using de-identified specimens, urine and saliva were collected from a small cohort; urine was spun and the pellet preserved in RNA-later. Saliva was collected using the Oragene-RNA system. RNA quality was assessed with the Agilent 2100 Bioanalyzer.

Results: There were 12 buffy-coat (blood) specimens, 4 blood specimens with PAXgene, 5 urine specimens, and 5 saliva specimens. In the buffy coat specimens, 7/12 yielded "good" quality RNA, 1/12 yielded "adequate", and 4/12 yielded "poor" results. In the whole blood using PAXgene, 4/4 yielded "good" quality RNA. Among the two non-blood modalities, 3/5 urine specimens yielded "good" RNA quality, and 5/5 saliva specimens yielded "good" quality results.

Conclusions: In these preliminary analyses, the two systems that directly collected specimens (PAXgene for whole blood and Oragene for saliva) yielded "good" quality RNA for all specimens (9/9). The two systems that required an intermediary step (preparation of buffy coat for whole blood and placed in RNA-later, and preparation of urine sediment pellet and placed in RNA-later) tended to yield a lower rate (11/17) of "good" quality RNA ($p = 0.063$, Fisher's exact). While there may be several potential factors that contribute to these differences, systems that allow for direct collection of RNA in the field allow for higher quality RNA. (This work was supported by NIEHS grant UO1ES016003).

Comparison between SNPs found in prepubertal girls exposed to BBP and gene expression profile of BBP exposed rats during prepubertal period

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The most common type of genetic variation, a SNP (single nucleotide polymorphism) is defined as a single base change in a DNA sequence. SNPs may have etiologic importance if they occur in a significant proportion (e.g., more than 1 percent) of a large population. SNPs are scattered throughout the genome and are found in both coding and non-coding regions. SNPs can have silent, harmless, harmful, or latent effects. When present in coding regions, SNPs could alter protein structure and function, which in turn could influence a person's health.

We examined SNPs involved in obesity and neuro-endocrine pathways in the CYGNET Study, a longitudinal study examining predictors of pubertal maturation in girls recruited from the Kaiser Permanente Northern California membership. In this analysis of 397 prepubertal girls who tested positive for phthalates in the urine, SNP analysis was performed using DNA extracted from blood or saliva. The SNP data were obtained using a 48-snp platform with SNPlex genotyping technology, and body mass index (BMI) and breast stage development data were collected. The statistical analysis of the data was done using logistic regression models for SNPs as predictors of BMI (dichotomized at 85th national percentile) or breast stage (categorized as stage 2+ vs. stage 1). The SNPs were examined both as 3-level variables (homozygous variant, heterozygous, homozygous wild type (wt)) and as 2-level variables (any variant allele vs. wt).

Results show that SNPs in several genes (e.g.: peroxisome proliferator-activated receptor delta (PPARD) or glutamate decarboxylase 1 (GAD1)) were associated with either BMI or breast stage development. When the same genes were searched in the gene expression arrays performed in the mammary gland of rats exposed prepubertally to benzyl butyl phthalate (BBP), an association with their dysregulated expression was found.

These results suggest that the increased BMI and advanced breast development in prepubertal girls is related in part to genes involved in lipid metabolism and obesity, and that these genes may be perturbed by exposure to BBP. While these data must be considered with caution, they highlight an important area that deserves further exploration and research. (*This work was supported by NCI and NIEHS Grants U01 ES012771 and U01 ES12801*).

Gene expression changes induced by DEHP (diethyl hexyl phthalate) in mammary gland, blood and buccal mucosa of 35 days old rats

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The objective of this study was to determine if genomic changes induced in the mammary gland of rats exposed to endocrine disruptors such as DEHP (diethyl hexyl phthalate) are also expressed in the blood and the buccal mucosa of the same animal. The rationale for this protocol is based on the need to translate this approach to humans in which exposure could be evaluated in the blood and buccal mucosa as a reflection of their effect in the breast tissue.

For this purpose lactating Sprague Dawley rats were gavaged on days 2-20 postpartum with 0 and 250 mg DEHP/kg BW on days Monday – Friday to effect lactational exposure of the offspring. The female offspring were sacrificed at time of weaning (21 days post-partum), at 35 days (shortly after puberty), and at 50 days post-partum. Control animals were treated with sesame oil. Each group contained 6 samples. The mammary glands were dissected from live anesthetized animals to minimize oxygen depletion and proteolysis. Blood was obtained *via* cardiac puncture and immediately placed into PAXgene blood RNA tubes. Buccal mucosa was obtained *via* cheek swabs and kept frozen until the time of RNA extraction. In the present work we explored the gene expression changes induced by DEHP in 35 days old rats using Agilent Microarrays. Using 200ng of total RNA from blood, buccal mucosa and mammary gland tissue, we found that the rat Agilent platform of 60-mer oligo-microarrays (4x44k) was outstanding in quality and reproducibility. Fisher's exact test was used to assess the statistical significance of the comparisons.

Our results show that 123 probes were differentially expressed among the three different tissues tested (57 genes differentially expressed between Blood and Buccal Mucosa, 34 genes differentially expressed between Mammary Gland and Buccal Mucosa, and 32 genes differentially expressed between Mammary Gland and Blood). Two common genes were found to be differentially expressed in the three tissues at 35 days post-partum in rats treated with DEHP

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The Effect of Exposure to BPA, BBP or TCDD on Rat Mammary Gland Fatty Acid and Lipid Metabolism

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Bisphenol A (BPA), BBP (Butyl benzyl phthalate) and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) are endocrine disruptors that may be involved in the genesis of hormone-dependent cancers, including that of the breast. To determine whether exposure to these agents alters the expression of genes that are directly or indirectly influencing fat and cholesterol metabolism, we systematically explored these metabolic pathways in female rats that were exposed to BPA, BBP and TCDD at prenatal or prepubertal stages of the development.

RNA was extracted from mammary tissue and used in whole genome Agilent microarrays. Gene expression of treated animals was compared to control using Empirical Bayes t-test. After data analysis, the expression values were plotted in a heat map for visualization of pattern of modulation of genes that directly or indirectly influence fat and cholesterol metabolism.

Changes were found in both TCDD and BBP, however, the former had more significance impact in the fat and cholesterol metabolism. BPA had little effect. The predominant changes were found in the animals exposed to TCDD at prepubertal age. A pathway analysis for prepubertal treatment with TCDD at 35 and 50 days showed that many lipid metabolism related genes were up-regulated. Also pathways such as citric acid cycle, butanoate, propanoate, valine, leucine and isoleucine degradation pathways were enriched. Important genes for fat and cholesterol metabolism, such as PPARG, PPARD, START, and LIPE were also up-regulated. A set of up-regulated genes was analyzed using IPA (Ingenuity Pathways Analysis) to identify any functional networks that might provide further clues to their involvement in fat metabolism. The most important genes in this cluster were PPARG and PPARD, which are part of the PPAR complex that along with RXRA activates various genes. Up-regulation of PPARG and PPARD activates genes involved in fatty acid uptake (CD36, FATP), lipoprotein metabolism (LPL), mitochondrial β -oxidation (FASN), peroxisomal β -oxidation (ACOX) and many other genes responsible for inflammation and growth hormone homeostasis. Interestingly, certain up-stream regulator genes for PPARA/RXR pathway such as Adiponectin and IRS1 were also up regulated.

The data clearly indicate that TCDD has a strong effect in deregulating the expression of genes involved in fat and cholesterol metabolism. (supported by NIEHS Grant UO1-276671 NCI-NIEHS Grant UO1 ES012771).

Influence of Fatty Acid Diets on Gene Expression in Rat Mammary Epithelial Cells

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Background: Dietary factors have been implicated in modifying the susceptibility to breast cancer initiation and promotion.

Objectives: The goal of the study was to examine the impact of dietary fatty acids on the gene expression levels in the developing mammary epithelium before and during puberty.

Work Performed: The diets contained of n-9 monounsaturated fatty acids (olive oil), n-6 polyunsaturated fatty acids (safflower), or saturated acids (butter). Female Sprague Dawley rats were placed on high (40%) or low (10%) fat diets for 30 days, bred, and their offspring were examined for dietary-induced changes in gene expression in the mammary ductal epithelial cells at day of weaning (21 days) and at the end of puberty (50 days after birth). Independent samples coming from a single pup from five to six different dams for each diet were collected by LCM isolation of mammary epithelial cells. Gene expression levels were assessed by hybridizing labeled RNA from each pup to the Affymetrix whole genome GeneChip.

Results: Using a stringent statistical significance threshold, 44 genes at day 21 and 90 genes at day 50 were differentially expressed compared to the reference diet from a total of 11,498 genes examined. The most highly significantly enriched gene ontology for both age groups was "Mitotic Cell Cycle" ($fdr < 5 \times 10^{-5}$ at day 21, and $fdr < 4 \times 10^{-17}$ for day 50). 28 of the 90 differentially expressed genes at day 50 and 12 of the 44 differentially expressed genes at day 21 were cell-cycle related. The highest scoring networks constructed by Ingenuity Pathway Analysis for the two groups were also highly enriched for the cell cycle and cancer related genes (p -value $< 10^{-46}$ for 21 day and p -value $< 10^{-62}$ for 50 day genes). 4 of the day 50 genes were also involved in the IPA G2/M check point canonical pathway. Cluster analysis yielded three distinct functionally coherent clusters. An immune cluster of 362 genes included 49 immune system related genes, a steroid cluster of 50 genes included six sterol biosynthesis related genes and a cell cycle cluster of 426 genes contained 62 cell cycle related genes.

Conclusions: Dietary fatty acids upregulated expression of proliferation genes above the normal physiological level. The combination of higher cellular proliferation compared to the normal level and the dietary-induced cell cycle check point pathway suggest an increase in DNA damage specific to mammary epithelium and thus, a potential increase risk of mammary cancer in later life. *Supported by grants U01ES12770 and R01HG003749.*

Global gene expression changes induced in the mammary gland of animals exposed to BPA, BBP, and TCDD at different developmental stages.

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Xenobiotics such as Bisphenol A (BPA), n-benzyl butyl phthalate (BBP) and 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) are endocrine disruptors that can affect the development of the mammary gland.

This study aims to investigate whether exposure to these compounds at different stages (prenatal, prepubertal) and dose levels (low, high), affects the global gene expression profile of the rat mammary gland at different ages.

To examine the significant biological functions represented by the genes differentially expressed between exposed and non-exposed rat mammary glands at 21, 35, 50 and 100 days we used Gene Ontology (GO) annotations, that describe group of genes in terms of their associated biological processes. Conditional hypergeometric tests, as implemented in the Bioconductor package, *GStats*, were performed to evaluate the overrepresentation of GO terms among differentially expressed genes. For further analyses, GO terms were manually combined according with their commonality of the biological functions in a variety of groups, such as transcription and DNA related genes, oncogenes, tumor suppressor genes, DNA damage response and repair genes, apoptosis, neurotransmitter genes, immunity and inflammation genes, fatty-acid and lipid metabolism, and cell differentiation and development genes.

No significant overlap of enriched GO terms among these xenoestrogenic compounds was found, suggesting that these endocrine disruptors act upon different processes. Of great interest was the observation that the transcription and DNA related genes, including estrogen and DNA damage response signaling pathways, cell differentiation and development genes were mainly affected upon BPA action. Over 550 tumor suppressor genes were tested in the different experimental conditions and we found that TCDD had the greatest effect on the tumor suppressor genes. Several genes related to the fatty acid and lipid metabolism were up-regulated by both BBP and TCDD treatments, but not by BPA. Our data demonstrate that each xenobiotic induces a set of specific changes in the mammary gland that may lead to different biological responses.

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Notch and wnt/ β -catenin signaling pathways in the mammary gland are dysregulated by prepubertal but not by prenatal exposure to BPA and are determinants of the increased susceptibility to DMBA-induced carcinogenesis.

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Bisphenol A (BPA) may contribute to the development of hormone-dependent cancers, such as those originated in the breast. Our Center has previously reported that whereas prepubertal exposure to BPA increased DMBA (dimethylbenz[a]anthracene) induced tumorigenesis in 50 days old rats, prenatally BPA exposed animals did not. The objective of this study is to determine the genomic basis that could explain this different susceptibility to carcinogenesis. For this purpose we have analyzed the gene expression profile of the mammary glands of 50 days old rats exposed prenatally or prepubertally to BPA (*Journal of Endocrinology* 196: 101-112, 2008). Gene expression of treated animals was compared to control using Empirical Bayes t-test. Genes with at least 1.2 fold difference in at least one condition and q-value<0.05 were considered differentially expressed. We found about 1200 genes differentially expressed, being the modulation of these genes more accentuated in the prepubertal group. Gene ontology (GO) analysis demonstrated that genes that were upregulated by any of the treatments affected GO terms related to cell nucleus, whereas the downregulated genes mainly affected those related to extracellular environment. Besides the changes common to both periods of exposure, prepubertal treatment showed additional effects at the level of developmental processes. Analysis of canonical pathways demonstrated that estrogen receptor and aryl hydrocarbon receptor signaling and xenobiotic metabolism were enriched by the upregulated genes in all conditions. However, two important pathways that are related to neoplastic transformation, notch and wnt/ β -catenin signaling, were affected only by genes modulated in the prepubertal treatment. The data provides genomic basis for the increase in tumorigenic response to DMBA in animals exposed prepubertally to BPA. (Work supported by NCI and NIEHS Grant UO1 ES012771).

Prenatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin significantly affects the expression of immune response genes in the adult rat mammary gland.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a dioxin produced through combustion processes such as waste incineration. Humans are exposed to TCDD by consumption of contaminated food and beverages, inhalation, or absorption through the skin. TCDD accumulates in plant tissues and animal fat, inducing both acute and chronic effects.

Our study is aimed at determine if prenatal exposure to TCDD has a long-term effect on the gene expression profile of the mammary gland during its development.

For this purpose, pregnant Sprague-Dawley rats received 3 μ g TCDD/kg BW of or equivalent volume of sesame oil (control group) at day 15 post-conception. When the offspring reached 21, 35, 50 or 100 days old, females were euthanized and the abdominal mammary glands were extracted for RNA isolation. Gene expression screening was performed using Agilent 22K cDNA microarrays. Statistical analysis was conducted using GeneSpring v9.0 software; probes with p<0.01 and at least 50% fold change difference were considered differentially expressed.

Prenatal TCDD exposure resulted in dysregulation of 87, 42, 20 and 1816 gene transcripts at 21, 35, 50 and 100 days of age, respectively. The genes found to be dysregulated varied at each specific age. Of great significance was that the mammary gland of the 100 days old animals had the highest number of genes affected by the treatment. Enrichment analysis demonstrated that the dysregulated genes were related to cell death, cancer, immune response, immunological diseases, cell growth and proliferation. Interestingly, 10 of the 13 canonical pathways observed in this group were immune related, and most of the genes belonging to these pathways were down-regulated by TCDD. Some of them were signaling pathways for leukocyte extravasation, B and T cell receptors, interferon, and natural killer cells. Our data allowed us to conclude that TCDD affects the gene expression profile of the mammary gland and that the effect is observed long after the prenatal exposure. Of significance is that the depression of the immunosurveillance pathway in the mammary gland may negatively affect the susceptibility of this organ to carcinogenesis.

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Different patterns of oxidative metabolism of MEHP to MEHHP, MEOHP and MECPP

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Background: Di(2-ethylhexyl) phthalate (DEHP) is commonly used as a plasticizer in polyvinyl chloride (PVC) plastics to provide flexibility, strength and optical clarity. PVC plastics are used in household products such as imitation leather, rainwear, upholstery coverings, shower curtains, food packaging materials and children's toys. In animal studies, Mono(2-ethylhexyl) phthalate (MEHP), the primary metabolite of DEHP, has been found to have significant effects on hepatic function in animal studies, and to decrease androgen activity in male infants. MEHHP is the oxidative metabolite of MEHP, and MEOHP and MECPP are secondary oxidative metabolites.

Hypothesis: Differences in activity of enzymes in the phthalate metabolism pathway will affect the ratio of primary metabolite, MEHP, to secondary and tertiary metabolites.

Methods: In a study of pubertal maturation, we measured urinary concentration of phthalate metabolites of young girls (age 6-7) in greater Cincinnati, including MEHP and some of its oxidative metabolites. Concentrations MEHP were compared to its oxidation products, across two racial categories.

Results: Measurements of urinary phthalate metabolites were available for 322 girls, but for the analysis of subgroups defined by the ratio MEHP:MEHHP, we used only the data of those with values >LOD for both metabolites, and only Caucasian and African-American girls (N=252). The median ratio of MEHP:MEHHP was 0.079 (or 1:12.7) with a right skewed distribution. We created subgroups of those with MEHP:MEHHP <0.140 (or > 1:7.1, N=208, group A) and ≥ 0.14 (or $\leq 1:7.1$, N=47, group B). The median value for urinary MEHP was larger in group B (5.8 vs. 3.3 ug/gCr), but the median value for all other measured downstream metabolites was 75-85% greater in group A vs. B. Of the 82 African-American (AA) girls, 24.4% were in group B vs. 15.6% of the white girls. In subgroups defined by the ratio of the secondary oxidative metabolites, girls with relatively more urinary MECPP (N=68) had a lower median value of both MECPP (53.6 vs. 64.0 ug/gCr) and MEOHP (11.7 vs. 26.6 ug/gCr) than the remainder of the cohort (N=254).

Conclusions: The distribution of ratios of MEHP to MEHHP suggests difference in cytochrome p450 activity in population subgroups, potentially representing genetic variants. Studies in animal models suggest that the

primary metabolite, MEHP, has more physiologic activity and potentially greater endocrine disruptor capacity, suggesting that blacks may be at greater risk of endocrine disruption from exposure to phthalates.

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Community Outreach: High School Students involved in Breast Cancer Research

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The Great Neck Breast Cancer Coalition provided scholarships to two Great Neck high school students with aspirations to increase participation and communication between scientific laboratories and the Great Neck community. In order to raise awareness about the scientific methods used in prominent laboratories and about the environmental causes of breast cancer, both students spent two weeks at the Dr. Ana M. Soto Laboratory in Tufts University in Boston. There, they participated in research involving endocrine disrupting compounds (EDC's) and learned how common chemicals can have a profuse effect on hormone levels in women. Particular emphasis was given to the chemical Bisphenol-A (BPA), an estrogen-mimicking chemical that is found in our environment due to plastics manufacture and industry. The two students learned that even low exposure of uteri to BPA can negatively affect hormone levels and future mannerisms of rats and mice. These changes in estrogen levels caused tumors and lesions in the mammary glands and tissues. Scientists in the Soto lab and other labs have hypothesized and shown through extensive experimentation that fetal mouse and rat exposure to BPA can disrupt mammary gland development and cause an increased risk of mammary tumors in mice and rats. These results may be parallel in humans, and may explain the recent increased incidence of breast cancer in the female population in the United States.

In addition to learning about EDC's and BPA, both students actively participated in research done by the scientists at the Ana M. Soto lab. Although scientific discoveries were not part of the two-week program, both students were engaged in the everyday procedures of the lab, and learned about the daily processes and methods of research. The program helped the students learn that scientific research is not at all unreachable by the public, and in fact scientific researchers encourage community participation. This program also encouraged students to take initiative in exploring Boston. On the weekends, the students could shop, visit museums, or simply tour Boston. The program taught students the responsibilities of living alone. This is a summary of their experiences at the Soto Lab.

Project funded by two scholarships granted by the Great Neck Breast Cancer Coalition, Great Neck, New York 11023.

The effect of prepubertal exposure to Bisphenol A on rat mammary gland morphology and gene expression.

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Bisphenol A (BPA), formerly called 4,4'-dihydroxy-2,2-diphenylpropane, is a commonly used chemical found in re-microwaveable baby bottles and the polycarbonate lining of plastic bottles. BPA is found in normal levels in the environment, mostly as a byproduct of industrial processes. Studies have shown that the levels of BPA found in the environment are non-hazardous to human health. However, research also suggests that BPA could lead to breast cancer since BPA is an endocrine disruptor, meaning it can mimic hormones that have a functional role in the body.

Due to these properties it was of interest to determine whether BPA could alter the terminal end buds (TEBs) structures of the mammary gland that are the target of carcinogenesis. For this purpose we have analyzed the effects of prepubertal exposure to BPA on the 50 days old rat's mammary gland.

Nursing rats received, through gavage, 250 µg/kg body weight of BPA, during 21 days from the delivery to weaning. The female offspring were sacrificed when they reached 50 days of age. Their abdominal mammary glands were extracted and used for whole mount preparation for TEB counting, or cDNA-microarrays gene expression analysis.

The results indicated a slight decrease in the number of TEBs in mammary glands of the treated rats when compared to the control group. However, gene expression analysis revealed changes in expression of numerous genes reported in breast cancers, such as *Vav2*, *Nfkb1*, *Tnsrf11a*, and *Mycn*. We have concluded that although prepubertal exposure to BPA does not affect significantly the rat mammary gland morphology of 50 day old rats when compared to the control group, the compound has an effect on the mammary gland gene expression. BPA altered the expression of some genes that have been reported in primary breast cancer as well as in the inflammatory type.

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Stat5a is Required for Branching During Early Mammary Gland Development

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Signal transducer and activator of transcription (Stat) 5a is a critical regulator of mammary gland development, and its importance in the late pregnant and lactating gland is well established. However, although active Stat5a is present in mammary epithelial cells in pubertal and adult virgin mice, little is known about its function during early mammary gland development. We previously demonstrated that Stat5a expression is induced at puberty by the ovarian hormones estrogen (E) and progesterone (P), and hypothesized that it is an important mediator of the effects of these hormones on the developing gland. In the current research, we tested this hypothesis by comparing mammary gland morphology, and the response to E+P treatment, in wild type and Stat5a^{-/-} mice. The mammary glands of Stat5a^{-/-} virgin mice exhibited defects in primary, secondary and side-branching, indicating that Stat5a is required for normal branching. This finding is consistent with our hypothesis, since branching is known to be dependent on E+P. To directly examine the role of Stat5a in the response to these hormones, wild type and Stat5a^{-/-} mice were ovariectomized, then treated with E+P or control vehicle. The mammary glands of Stat5a^{-/-} mice displayed an attenuated proliferative and morphological response to E+P, confirming that Stat5a is an important mediator of the effects of these hormones. Finally, we identified a downstream target of Stat5a, receptor activator of NF- κ B ligand (RANKL). In wild type mice, RANKL was induced in response to E+P treatment, but Stat5a^{-/-} mice exhibited a defect in this response. RANKL is a secreted protein that acts in a paracrine fashion by binding to its receptor on target cells. One such target is the inhibitor of DNA binding (Id) 2, which translocates to the nucleus in response to RANKL binding to its receptor. Nuclear translocation of Id2 in response to E+P treatment was also diminished in Stat5a^{-/-} mice. Together, our data suggest that a pathway in which Stat5a induces RANKL, which then causes nuclear localization of Id2, mediates at least some of the effects of E+P in the developing mammary gland.

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Latina Adolescent Outreach Project

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Background: The need for accurate, developmentally appropriate information for adolescent girls specific to breast cancer risk factors and preventive health is a topic of keen interest to breast cancer advocates and researchers (1). Latinos represent the fastest growing ethnic demographic group in Marin County and the Bay Area, consistent with California and national trends (2). Latinos encounter unique cultural and linguistic barriers to health information and cancer prevention services (3).

In an effort to modify the *Breast Cancer and Environment – Peer Education Tool Kit* (4) for a more diverse adolescent population, Zero Breast Cancer and bi-cultural advisors from the University of California San Francisco engaged local Latina adolescents in the process of adapting and translating key components. The Latina Adolescent Outreach Project has produced a culturally relevant brochure entitled “*Breast Cancer Tips for Latina Teens, Young Women and Families.*”

Objectives: The primary objective was to culturally adapt and translate into Spanish three components of the *Breast Cancer and Environment – Peer Education Tool Kit* for Marin County Latina adolescents: Top Twelve Messages, Teen Brochure, Personal Action Plan. The secondary objective was to disseminate the adapted, translated materials to high school health educators and community organizations.

Methods/Work Performed: ZBC and UCSF engaged the Novato Youth Wellness Collaborative, a consortium of community health organizations, in the project planning and implementation. In an effort to understand Latina adolescents’ perceptions and attitudes related to breast cancer risk reduction and *Tool Kit* adaptation, we conducted two 90-minute focus groups with Latina adolescents from Novato, California, aided by a bi-lingual health educator.

- Participant recruitment was accomplished through a bilingual announcement
- Parental consent was obtained
- Group facilitators conducted reviews of *Tool Kit* components, probing students’ information needs, cultural themes and language preferences
- A consensus process was employed to re-design the *Tool Kit* messages, brochure, and personal action plan
- A professional service produced the Spanish translation

- A graphic designer incorporated Latina images into the brochure
- The Breast Cancer Coordinating Council (Marin County Department of Health and Human Services) was consulted for Latina oriented resources
- We followed a standard qualitative approach to review focus group data

Results/Discussion: The new brochure, *Breast Cancer Tips for Latina Teens, Young Women and Families*, integrates culturally grounded terminology and graphics to convey breast cancer educational content for Latina adolescents. The brochure (available at BCERC poster session) may be used in high schools and community health programs serving Latina adolescents and women. The brochure encourages teen and family discussions of breast cancer factors and risk-reduction practices, including information on environmental exposures and prevention resources.

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Differential Effects of Peripubertal Exposure to Perfluorooctanoic Acid on Mammary Gland Development in C57Bl/6 and Balb/c Mouse Strains

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Perfluorooctanoic acid (PFOA) is a persistent environmental contaminant commonly used in manufacturing fluoropolymers that are widely used in industrial and consumer goods. Due to its persistence, there has been growing concern regarding its toxic effects. Recently, the Breast Cancer and Environment Research Centers Epidemiology Project identified a subgroup of girls in the Cincinnati cohort that had elevated levels of PFOA in their serum samples. Studies have shown that exposure of rodents to PFOA during pregnancy results in developmental toxicity in their offspring; however little is known about the effects of peri-pubertal PFOA exposures.

To investigate the effects of peri-pubertal PFOA exposure, three-week-old C57Bl/6 and Balb/c female mice were orally dosed with either 0 (control), 1, 5, or 10 mg PFOA/kg body weight, daily for four weeks. At the time of sacrifice, livers, uteri and mammary glands were collected for histological evaluation. The 10 mg/kg PFOA dose resulted in a significant decrease in body weight in both strains. Furthermore, PFOA treatment significantly increased relative liver weights and caused hepatocellular hypertrophy in both strains in a dose-dependent manner. However, the severity of the lesions in C57Bl/6 mice was more pronounced. PFOA had significant stimulatory effects on the uterus of C57Bl/6 mice given 1mg/kg PFOA which coincided with increased uterine size and weight, and well-developed endometrial lining, glands and stroma. Conversely, effect of PFOA on the uteri of Balb/c mice was inhibitory. While PFOA had dose-dependent inhibitory effect on mammary gland development in Balb/c mice, C57Bl/6 mice given 5 mg/kg PFOA exhibited an increased number of terminal end buds and enlargement of duct ends.

Collectively, these results illustrate that peri-pubertal PFOA exposure has significantly different effects on the uteri and mammary glands between mice strains. In C57Bl/6 mice, PFOA is stimulatory in the uterus and mammary gland, however in Balb/c mice, PFOA is inhibitory. Therefore, caution needs to be taken when interpreting the effects of PFOA in a single mouse strain.

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Epigenetic marks associated with early-life exposure to high dietary fatty acids in rat mammary epithelial cells

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Background: Population studies suggest a link between high fat consumption and increased breast cancer risk; yet the mechanisms underlying this connection remain elusive. Data are particularly lacking on the impacts of early-life exposure and differential risks of different fats.

Hypothesis: We here test the hypothesis that the exposure of dams to different high-fat diets induces unique epigenetic marks in mammary epithelial cells of their offspring before weaning.

Methods: Female Sprague Dawley rats were placed on high-fat diets (40%) for 30 days, bred, and their offspring killed on day 21. Four dietary regiments were used: high olive oil (HFO; primarily n-9 monounsaturated fatty acids), high safflower oil (HFS; primarily n-6 polyunsaturated fatty acids), or high butter (HFB; primarily saturated fatty acids), and a reference diet (AIN-93G). Mammary ductal epithelial cells were obtained using laser capture microscopy-assisted microdissection. Differentially methylated DNA sequences associated with one or more high-fat diets were identified by methylation-sensitive restriction fingerprinting. Bisulfite sequencing analysis was used to characterize changes in methylation patterns in candidate sequences. Real time RT-PCR was employed to quantify transcript levels.

Results: Over 20 sequences were identified to be differentially methylated in mammary epithelial cells obtained from offspring exposed *in utero* and during lactation to high-fat diets when compared to those exposed to the reference diet. Four sequences were found to be homologous to known genes and located in their 5'-CpG island(s): LINE-1 type transposase domain containing 1" (*L1td1*), solute carrier family 12 (sodium/potassium/chloride transporters), member 2 (*Slc12a2*), solute carrier family 6 (neurotransmitter transporter, taurine), member 6 (*Slc6a6*), and hippocampin-like 1 (*Hpcal1*). Exposure to HFB induced hypermethylation of *L1td1*, *Slc12a2* and *Hpcal1* but not *Slc6a6*. HFO affected only *Hpcal1* and *Slc12a2* whereas HFS had minimal effects. With regard to the expression of these genes, HFO and HFB had the most marked influences on the silencing of these genes.

Conclusion: These data provide the first evidence that dietary fatty acids are mother-to-fetus active compounds that could cause unique epigenetic marks in mammary epithelial cells of offspring. Ongoing experiments focuses on seeking functional relationships between these epigenetic changes and breast cancer risk.

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Phthalate Metabolites and Body Size Characteristics in Urban Minority Girls

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According to the CDC, the prevalence of overweight among U.S. children aged 6-11 years has risen from 6.5% in 1976-1980 to 18.8% in 2003-2004. Overweight children are at increased risk of becoming overweight adults which in turn puts them at increased risk for many chronic diseases including diabetes, heart disease and cancer. Energy imbalance (more calories consumed than used) is considered the main contributor to becoming overweight. However, genetics and environmental factors may also play a role. Phthalates, chemicals found in many consumer products, flexible plastics and fragrances, have the potential to disturb endogenous hormonal levels and may influence the development of obesity by affecting insulin sensitivity. A recent report using the NHANES 2001-2002 cross-sectional data in men supports this hypothesis. Given the increasing rates of overweight and the ubiquitous exposure to phthalates, we are investigating the relationship between phthalate exposure and body size characteristics among young girls.

Methods: Growing Up Healthy (GUH) is a prospective cohort study of Hispanic and Black, New York City girls between 6 and 8 years old. GUH is part of a NIEHS/NCI funded consortium (Breast Cancer and the Environment Research Centers) of three centers across the U.S. At baseline, parents/guardians were interviewed in-person, in either English or Spanish, about the girls' environmental exposures, physical activity, medical history, and demographics. Anthropometric measurements (weight, height, waist (WC) and hip circumference (HC)), using a standardized protocol, were made by trained interviewers.

Body mass index (BMI: weight/height²) and BMI percentile (CDC age and gender specific) were calculated. Casual urine samples provided by each girl were analyzed by the Centers for Disease Control and Prevention for a panel of environmental exposure biomarkers, including 9 phthalate metabolites.

The molar sums of low and high molecular weight phthalate metabolites, (LoMWP:mEP, mBP, miBP and HiMWP:mBzP, mCPP, mEHP, mEHHP, mEOHP, mECP, respectively) were calculated. Cross-sectional analysis using baseline data was conducted for this report. Age and race-ethnicity adjusted geometric means for each phthalate metabolite as well as LoMWP and HiMWP were compared between girls in the upper quartile of each body size characteristic (BSC) and those in the combined lower 3 quartiles using generalized linear models. Adjusted geometric means (ng/ml or nmoles/ml) and 95%CI are presented.

Results: Data from 398 girls were available for analysis from 412 girls enrolled at baseline. Adjusted mEP geometric means (BSC Q4 vs. BSC Q1-3) were significantly higher for BMI [271.2(176.2-417.5) vs. 155.7(127.2-190.7); p= 0.05], HC [275.6(186.4-407.6) vs. 154.3(127.0-187.5); p=0.02] and WC [291.5(196.8-431.8) vs. 151.9(125.3-184.3); p=0.01]. Similar differences were observed for the LoMWP, primarily due to mEP's contribution to this sum. Adjusted HiMWP geometric means were elevated for girls in the upper quartile of HC and WC [1.198 (0.833-1.723) vs. 0.764 (0.638-0.916) p=0.05; 1.342 (0.932-1.932) vs. 0.737 (0.616-0.882) p=0.01, respectively].

Discussion: These findings suggest that phthalate exposure may contribute to increased overweight in children. However given the cross-sectional design of this analysis, we expect that our longitudinal data, when it becomes available, will help to clarify this association.

An *In Vivo* Dendritic Cell Targeting Strategy for Treating Primary and Metastatic Breast Cancer Examined in a Stringent Animal Model

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Survival rates for patients with recurrent or metastatic breast cancers are poor, and currently available treatments have undesirable side-effects. Therefore, a therapy that effectively combats primary and metastatic tumor cells simultaneously, with fewer side-effects, is necessary. In this regard, genetic immunotherapy, which aims to stimulate patients' immune systems to successfully combat diseases, is a promising approach. As critical regulators of immune responses, dendritic cells (DCs) are attractive targets for such strategies. In the immature state, DCs monitor peripheral tissues, endocytosing proteins and processing them for antigen presentation to T cells. If DCs encounter a maturation stimulus, interacting T cells are prompted to instigate immune attacks, ridding the system of cells expressing these antigens. The presentation of antigens by DCs can thus be exploited to activate T cells specific for tumor-associated antigens (TAA), leading to clearance of tumor cells.

Vaccines composed of autologous DCs transduced with tumor-associated antigens (TAA) and matured *ex vivo* have been investigated as immunotherapeutic treatments for breast cancer in clinical trials. Unfortunately, responses in clinical trials have not matched the promising responses in mouse studies. Several observations could explain these results, including: 1) DCs matured *ex vivo* may not effectively mimic DCs matured *in vivo*, and 2) regulatory T cells (Tregs) activated by tumor-secreted cytokines may inhibit the anti-tumor immunity. Therefore, we hypothesize that delivery of TAA to DCs *in vivo*, coincident with Treg suppression, will stimulate proper DC maturation and allow a systemic immune response to successfully combat primary and metastatic breast cancer cells.

Adenoviruses (Ad) are well-suited to transfer TAA *in vivo*. Therefore, we developed strategies to target Ad5 to CD40 expressed by DCs. CD40-targeted Ad5 efficiently transduces DCs, leading to expression of TAA as well as DC maturation, *in vitro*. Preliminary *in vivo* vaccination

experiments in healthy dogs indicate that, compared to untargeted Ad5, CD40-targeted Ad5 elicits qualitatively enhanced TAA-specific T cell responses and a Th1-biased humoral response. We are currently optimizing vector dosages and examining potential synergistic effects of Treg suppression.

Mammary carcinomas are the most common cancer of un-spayed female dogs. Similar to human breast cancer, these tumors can metastasize, and recurrence is common. Unlike mice, dogs naturally develop mammary cancers, possibly due to exposure to similar environmental carcinogens as humans. Thus, dogs provide the opportunity to 1) evaluate therapies in an immunologically relevant model, 2) treat canine patients, and 3) facilitate rapid and more accurate clinical translation for breast cancer therapy.

Breast Cancer Metastasis Suppressor 1 (BRMS1) selectively modulates growth factor signaling without affecting chemosensitivity

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The metastasis suppressor BRMS1 halts ectopic growth (metastasis) without blocking orthotopic tumor formation. BRMS1-expressing tumor cells reach secondary site but do not colonize distant tissues. To explain this differential tumor growth, we hypothesized that BRMS1 selectively restricts the ability of tumor cells to respond to exogenous stimuli. Metastatic human breast cancer cells MDA-MB-231 (231) and MDA-MB-435 (435), lacking endogenous BRMS1 were stably transduced to express BRMS1 (231^{BRMS1} and 435^{BRMS1}). Cells were exposed to epidermal growth factor (EGF), hepatocyte growth factor (HGF) or platelet derived growth factor (PDGF), mitogens that promote breast cancer metastasis. Downstream effectors were analyzed by immunoblot. EGF-stimulated Akt-Ser⁴⁷³ was markedly reduced in 231^{BRMS1} and completely abolished in 435^{BRMS1}; however, BRMS1 had a minimal effect on p42/p44 MAPK phosphorylation. EGF-receptor mRNA and protein were reduced (231^{BRMS1}) or undetectable (435^{BRMS1}) concordant with reduced (>95%) EGFR promoter activity. In contrast, no reduction in HGF-induced Akt and MAPK activation was seen despite reduced PtdIns(4,5)P₂ and unchanged HGF-receptor (c-Met) levels. Consistently, HGF-induced intracellular calcium release, as measured by ratiometric calcium imaging, were similar between vector- and BRMS1-expressing cells. Interestingly, PDGF-induced intracellular calcium release was attenuated although PDGF-receptor expression was unchanged in BRMS1-expressing cells. In the presence of differential signaling, BRMS1-expressing cells were equally sensitive as parental cells to chemotherapeutic agents including paclitaxel, doxorubicin, 5-fluorouracil, and vincristine measured by clonogenicity assays. Permitting specific signals to transduce may ultimately determine tumor cell growth at ectopic v orthotopic sites and provide insights into the biology underlying metastasis.

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Evaluation of a Community-based, Participatory Research Approach in the Bay Area Breast Cancer and the Environment Research Center (BABCERC)

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Background: The literature suggests that a community-based participatory research (CBPR) approach benefits the translation of research to the community by fostering co-learning, ensuring projects are community-driven, increasing trust between the community and researchers, and increasing local relevance of findings. However, there are few standards for assessing the efficacy of community participation, few formal evaluations of the CBPR approach and little data about the adoption and effectiveness of CBPR within the context of a multi-site multidisciplinary center.

Objectives: To assess key stakeholders' perceptions of alignment between a planned CBPR effort and CBPR guidelines. Of particular interest was the determination of what worked and why, as well as remaining challenges in need of further evaluation to achieve CBPR goals.

Methods: The Community Outreach and Translation Core (COTC) of the BABCERC incorporated principles of CPBR developed by Green and colleagues. Key stakeholders were identified: the COTC, researchers, and community members from 3 Bay Area Counties (Marin, Alameda and San Francisco) with a focus on low income communities. Three representatives from each group were identified. The evaluation approach included: individual interviews, a focus group and a quantitative rating of CBPR experiences. The rating instrument included questions adapted from 4 areas of CBPR: participants' involvement; impact on shape of the purpose and scope of the BABCERC study, implementation and context; and interpretation of research outcomes. Nine of 12 individuals representing each of the 3 key stakeholder groups completed the quantitative rating form, 12 participated in an individual interview and a subset of respondents participated in a focus group. Data were collected over a 5 month period in 2007. Data quality was assured through a continual process of member checking and triangulation.

Results: Of the items analyzed, 40% noted high alignment; 25% noted moderate alignment and 16% of respondents noted low alignment with CBPR principles; 19% were "not sure". Among the 4 areas of interest, participants had the most positive perceptions related to "participants and nature of their involvement" as well as "shaping the purpose and scope of the BABCERC". Greater variance in responses occurred relative to

"research implementation and context" and "nature of research outcomes". Recurring themes from the interviews and focus group included the importance and benefit of having COTC providing input into the research and the importance of annual town hall meetings for translation and dissemination of information. Several participants perceived a lack of clear agreement about how dissemination of project findings would happen, thus creating uncertainty. Further, there was concern as to the actual impact CBPR had on framing the research questions, particularly in the basic science studies; the level of scientific expertise needed to understand the research outcomes; and the differing priorities among issue-focused activists, the publication demands of the researchers and the community's interest in the practical application of research results. The challenge of communication across the 3 counties was also noted.

Conclusions: CBPR is effective in bridging gaps in knowledge and communication among scientists, advocates and the lay community. The translation and dissemination of science to the public, increased public understanding of the scientific process and the opportunity to build trusting relationships among diverse stakeholders are key benefits realized by incorporation of CBPR in the BABCERC study. However, given the small cross sectional sample and somewhat low community members' response to the quantitative survey in this work, it is clear that continuing effort is needed to validate the efficacy and effectiveness of CBPR.

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Light-induced circadian disruption alters clock gene expression in Sprague Dawley rat mammary gland and liver.

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There is evidence that alterations in the circadian rhythm have a role in cancer development. Since functional clock genes exist in liver and mammary tissue and could influence the risk of cancer development via light-induced circadian disruption, we investigated if the expression of certain clock genes is affected by the type of light used during the light cycle or by the disruption of the regular pattern of light and dark cycles.

To investigate lighting's role, female Sprague Dawley rats were exposed to different light treatments: a group exposed to a light/dark cycle of 12 hours each (LD), using 3500K fluorescent lamps (~70 μ W/cm²) as control group. A second group, exposed to a LD cycle using light-emitting diodes (LEDs, peak wavelength 525 nm, ~5 μ W/cm²), was identified as entrained group. A third group (disrupted) where the light cycle was reversed every 2 days using LEDs. After 14 days, rats were sacrificed every 4 hours during a 24 hour period. RNA was extracted from mammary gland and liver, and real time RT-PCR was utilized for analyzing the circadian rhythm related genes *Per-1, 2, 3, clock, Cry-1* and *I, bhlhb 2* and *bhlhb 3*.

All the clock genes exhibited a similar rhythmic expression between the control and the entrained groups; compare to those groups the disrupted one had a rhythmic expression but both the peak and the trough gene expression were delayed 8 to 12 hours. The clock genes maintained a rhythmic but different expression between the mammary gland and the liver tissues, except by *Per 3* and *Cry 1*, in which the peak and trough levels of expression were similar between both tissues of the same gene.

Our findings indicate that changes in the pattern of light in the environment affect the level and rhythm of expression of clock genes in both the mammary gland and liver. Because clock gene expression is an important factor in cell cycle regulation and development, these observations support the hypothesis that alterations in circadian rhythm could have implications in the etiology of breast cancer.

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Mammary Cancer Chemoprevention with the Polyphenol Resveratrol

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Despite recent advances in therapeutic treatments, breast cancer remains a serious disease and a leading killer among female cancers. In 2008, there will be more than 200,000 new cases of breast cancer and 40,000 deaths. There is, and should be, a strong effort to work toward the prevention of this disease. It is now well accepted that environmental factors, especially diet and lifestyle, play a critical role in determining one's risk for breast cancer. One dietary polyphenol that has received much attention for its health benefits, including anti-cancer properties, is resveratrol, a phytoalexin found in red grapes and red wine. We hypothesized that resveratrol given in the diet would protect against chemically-induced mammary cancer through mechanisms that involve mammary gland differentiation, epithelial cell proliferation, and apoptosis. Female Sprague Dawley rats were treated with 1000 mg resveratrol/kg AIN-76A diet from birth throughout life. At 50 days postpartum, animals were sacrificed to evaluate mammary whole mounts, cell proliferation, apoptosis, or treated with 60 mg dimethylbenz[a]anthracene (DMBA)/kg body weight to induce mammary adenocarcinomas. A follow-up tumor study with a lower dose of resveratrol (333 mg resveratrol/kg diet) was also done in the same manner. Both doses of resveratrol (1000 mg and 333 mg) were able to suppress mammary tumor multiplicity and increase tumor latency in a statistically significant manner. Mammary whole mount analysis revealed a modest increase in lobular structures, the least susceptible mammary terminal ductal structures to carcinogens. Cell proliferation and apoptosis assays revealed a significant decrease in mammary epithelial cell proliferation and a significant increase in apoptosis in mammary terminal end buds, the most susceptible structures to carcinogenesis in the mammary gland. As to the molecular mechanisms through which resveratrol may be protecting against mammary carcinogenesis and modulating mammary epithelial cell proliferation and apoptosis, we employed TaqMan low density arrays. We selected genes with a known role in mammary epithelial cell proliferation, apoptosis, steroid metabolism, and growth factors involved in normal and malignant mammary gland development. Exposure to resveratrol in the diet resulted in a significant decrease in gene expression of COX-1 and tended to increase the levels of PPAR- γ . Both of these genes can play a role in cell proliferation, apoptosis. We also observed a significant increase in the gene expression levels of caspases 2, 3, and 9, all of which are involved in the apoptotic cascade

and may help to explain the increased apoptosis that we observed with resveratrol in the mammary gland. We conclude that resveratrol in the diet can protect against mammary carcinogenesis by modulating mammary gland differentiation, and epithelial cell proliferation and apoptosis. (Supported by DOD-BC043793)

Development of a Tool to Test the Literacy, Health, Information, and Design Content of Websites

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Background: The internet is increasingly being utilized by people seeking health information. As a result, a myriad of studies regarding the evaluation of websites have been completed. However, the majority of these studies are superficial in depth by focusing mainly on design tenets, despite the fact that research has established the crucial role of other pertinent aspects of health websites, such as a variety of literacy issues, health content, and informational content.

Objective: In an attempt to launch a robust means to assess the plethora of issues surrounding optimal use of health websites, this study sought to: 1) develop four evaluation worksheets that measure design tenets, literacy issues, health content, and information content for health websites, including those for breast cancer, and 2) test the proposed evaluation methodology on five breast cancer websites.

Methods: An extensive literature review on website evaluation and the different types of literacy required to use health websites was performed. This review led to the creation of four evaluation worksheets for health websites, which assess: *basic design tenets* like proper format and usability; *health content* related to whether the website motivates users to perform health tasks and contains social support material; *literacy types* such as readability of the site and cultural diversity present on the site; and finally, *informational content* such as whether the site displays references and authors of material. The proposed evaluation methodology was then tested on five breast cancer websites, including: www.cbcpr.org, <http://www.intelihealth.com/IH/ihtIH/WSIHW000/8293/8293.html>, <http://www.lifetimetv.com/breastcancer/petition/signpetition.php>, http://www.drgreene.com/21_625.html, and http://cancer.uhc.edu/patients_families/treatment_types/breast/index.html.

Results: The five websites evaluated with this preliminary tool scored highest on the design tenets evaluation sheet while having very few of the assets required by the literacy, health content, and information content sheets.

Conclusions: In order to create more effective breast cancer websites, various types of literacy involved in using a website, information content, and health content need to parallel guidelines that have been identified by current research. This tool will allow organizations to first evaluate their website and then improve it through offering tailored guidelines based on responses given to the four areas of assessment.

Effects of Outreach on Retention Rates of Minority Children in a Longitudinal Study

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Background: The Growing Up Healthy (GUH) study is a longitudinal study of environmental exposures in low-income minority children. In order to promote study retention, education and outreach activities are offered to participants and their families by the Community Outreach and Translation Core (COTC) using a community-based participatory research framework.

Objectives: 1.) To determine the relationship between COTC activity attendance and study retention, measured as attendance at Year 1 follow-up. 2.) To determine the effects of targeted outreach efforts towards hard to reach participants.

Methods: A cross-sectional survey of parents of GUH participants presenting at the Year 1 follow-up health visit was used. The survey included questions about past COTC attendance, preferred activities, and barriers to attendance. COTC activity attendance was recorded at each event. The COTC activities for study participants included outdoor activities, science activities, and educational activities.

Results: Among 190 participants completing their first year in GUH, 48.9% attended at least one COTC event; 79.6% of COTC event attendees completed the Year 1 follow-up visit. The odds of attending the Year 1 follow-up were 4 times greater for COTC attendees than non-attendees. A significantly greater proportion of Latinos (56.3%) attended activities than African Americans (34.4%). The most popular COTC activities were giveaways followed by educational events, and outdoor activities. The least popular were the science activities. The most common barrier to attendance was time of the event. The most successful giveaway was the back to school backpack distribution event directly resulting in 62 follow up appointments. We utilized the popularity of the back to school backpack giveaway to reestablish contact and increase yearly follow up appointments amongst participants previously lost to follow up.

Conclusion: Results indicate that COTC efforts have been successful in retaining participation in the GUH study, characterized by a relationship between COTC event attendance and Year 1 follow-up attendance. Further insight into attendance patterns indicates that Latinos and African Americans have different attendance behaviors and time is a significant barrier to participation. The backpack distribution event increased retention rates with regards to follow up visits, especially amongst those participants previously lost to follow up. These findings will be used to modify future COTC activities in order to maximize outreach and retention.

The effect of prepubertal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on the rat mammary gland

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There is substantive evidence linking 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure to breast cancer. Previous studies have demonstrated that timing of exposure to TCDD greatly affects risk to develop tumors. In this study, we investigated the effect of prepubertal TCDD exposure on mammary gland development and genetic expression. Sprague-Dawley rats were orally administered either of two doses (6.67ng/kg of body weight or 20ng/kg bw) of 2,3,7,8-tetrachlorodibenzo-p-dioxin and sacrificed at 50 days of age. The fourth and fifth abdominal mammary glands were extracted and prepared for whole mount studies. Terminal end buds (TEBs) were then counted and expressed as a total number of TEBs per mammary gland. We found that rats that have received the higher dosage demonstrated a statistically significant greater amount of TEBs. In addition, branching and lobular differentiation were negatively affected in mammary glands of high-dose rats. Following morphological examination and gene expression analysis using cDNA- microarray, we found 472 transcripts over or under-expressed in the high-dose group, while 8 were over or under-expressed in the low-dose group. Among these genes, there are some that could potentially contribute to mammary carcinogenesis, including those involved in lipid metabolism, oncogenes, cell cycle, oxidative stress, and genes involved in cellular proliferation and apoptosis. Notably, *Cyp1b1* was the most upregulated gene in the TCDD-exposed animals at the two doses tested. Environmentally-activated *Cyp1b1* upregulation has been implicated in mammary carcinogenesis. In summary, our studies allow us to conclude that the animals treated with the higher dose of TCDD had an increase of TEBs and a significant alteration in gene expression. This could imply that TCDD exposure may create a cellular environment more suitable for increasing the risk of mammary carcinogenesis.

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Induction of myeloid-derived suppressor cells by tumor exosomes

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Myeloid-derived suppressor cells (MDSCs) promote tumor progression. The mechanisms of MDSC development during tumor growth remain unknown. Tumor exosomes have been implicated to play a role in immune regulation, however the role of exosomes in the induction of MDSCs is unclear. Here, we show that exosomes isolated from tumor (T-exosomes) are taken up by bone marrow myeloid precursors and switched the differentiation pathway of these myeloid precursors into MDS-like cells (CD11b+Gr-1+) that exhibit MDSC

functional and phenotypic characteristics including promotion of tumor growth. Furthermore, we identified that in vivo MDSC mediated promotion of tumor progression is dependent on T-exosome PGE2 and TGF- β molecules. T-exosomes can induce the accumulation of MDSCs expressing Cox2, IL-6, VEGF, and arginase-1. Antibodies against exosomal TGF- β and prostaglandin E2 (PGE2) block the activity of these exosomes on MDSC induction and therefore attenuate MDSC mediated tumor-promoting ability. Both exosomes PGE2 and TGF- β are enriched in T-exosomes in comparison with exosomes isolated from the supernatants of cultured tumor cells (C-exosomes). The tumor microenvironment has a great effect on the potency of tumor exosome mediated induction of MDSCs by regulating the sorting and the amount of exosomal PGE2 and TGF- β available. Together, these findings lend themselves to developing specific targetable therapeutic strategies to reduce or eliminate MDSC-induced immunosuppression and hence enhance host antitumor immunotherapy efficacy.

