The Mammary Gland as a Sensitive Tissue for Detecting Effects of Environmental Components

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The mammary gland of the rodent and human share many developmental, peri-pubertal, and differentiation landmarks, hormonal requirements for early proliferation, and tumorigenic processes and lesions. These commonalities and the short life span of rodents makes them a good choice for the study of environmental risk factors for breast cancer. Breast cancer research in the last few years has focused on early life exposures to low levels of endocrine disrupting compounds (EDCs), due to the identification of critical windows of prenatal, neonatal, and peri-pubertal exposure for persistent effects on mammary gland development of the adult rodent, and the lack of ability to identify environmental components of breast cancer risk in adult humans. Several EDCs have been identified that delay the morphological development of the rodent mammary gland following brief transplacental exposures. Among them are the high use herbicide, atrazine, and its biological metabolites. These compounds require only a 3 day exposure in late pregnancy for their effects in the offspring, and this window of sensitivity is still present during the early lactational period. When exposed to these compounds in a mixture similar in ratio and at a level that is 100 times higher than that detected in surface/ground water, delayed development of the mammary gland is still observed. However, while at higher concentrations this compound caused delayed vaginal opening (VO, a common sign of puberty in rodents), lower level exposures had no effect on VO. Nearly the same story can be told for the ubiquitous and persistent compound, dioxin. A single prenatal exposure to dioxin leads to delayed VO (with a lowest dose of 0.8 ug/kg), while doses as low as 0.2 ug/kg produce visible morphological changes in the mammary gland, and even lower levels lead to changes in biomarkers of exposure in this tissue. Recently, perfluorooctanoic acid (PFOA), a synthetic fluoropolymer used for water/grease/stain-proofing has been shown to cause delayed mammary gland development at prenatal exposure levels that do not affect VO or estrous cyclicity in rodents. Critics of using the mammary gland as a developmental landmark for puberty argue that tissue effects are simply due to effects of the EDC on the body weight of the animal. However, all three of the compounds described have effects on mammary developmental endpoints at maternal doses well below those needed to alter body weight of the offspring. One indicator of adverse effect of EDCs on mammary gland development is whether or not female offspring can nurse her own litter. All of the compounds described lead to altered F1 lactational gland development, which may decrease F2 postnatal pup weight gain, mortality, or both. Another indicator of adverse effect is tumor development – while dioxin has been shown to make the gland more susceptible to tumorigenesis during the elongated window of terminal end buds in the gland, atrazine and PFOA are still under investigation. *Disclaimer: This abstract and the corresponding presentation do not necessarily reflect EPA policy.*

Prenatal Exposure to Bisphenol A (BPA) Induces Genomic Alterations in the Rat Mammary Gland

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This study analyzes the genomic signature of the mammary gland of rats exposed prenatally to BPA. Pregnant Sprague Dawley rats received 25 or 250ug BPA/kg body weight (low and high dose groups) or equivalent volume of sesame oil (control group) from gestation day 10 through delivery. On days 21, 35, 50 and 100 of life, female litters from each group were sacrificed. 10 animals per group were used for RNA extraction for genomic signature screening using Agilent platform with 22,000 features. Statistical significance was determined based on a false discovery rate cut-off of 5% and fold change of 2 times. The genes were annotated according to known functions and canonical pathways. Based on the gene ontology the immune system and the immune lymphatic system development functions were overrepresented as a consequence of BPA exposure. The pattern of expression varies according to the dose. At the low dose the maximum level of genes expression occurs at 35 and 50 days of age with 30 genes and 26 canonical pathways including IL-10, apoptosis and chemokines signaling being affected. Inversely the mammary gland of the animals at 21 and 100 days of age were the most significantly affected by high dose exposure to BPA. 87 genes related to the immune system were affected by the BPA exposure at 100 days of age. These genes affected 62 canonical pathways; among them the IL-2, 4, 6 10, antigen presentation, apoptosis signaling, B cell receptor, chemokines, death receptor signaling, leukocytes extravasation, natural killer cells, NFβκ signaling, T cell receptor, TGFβ and VEGF signaling. BPA exposure during the development of the litter affects the gene expression of the immune system and lymphatic development. The high dose BPA treatment has an effect in a larger number of genes and extends the period of these changes to all lifespan of the animal, that can indicate a widening of the window of susceptibility to carcinogenesis.

Bisphenol A, Mammary Cancer and Proteomic Studies in a Rat Model

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Exposure to hormonally-active chemicals during critical periods of development are hypothesized to alter susceptibility for cancer. One such chemical, bisphenol A (BPA), is commonly used in the production of polycarbonate plastics, epoxy resins, and dental sealants. BPA has been shown to advance puberty and disrupt estrous cyclicity, identifying it as an endocrine disruptor. Our goal was to determine if BPA exposure can predispose for mammary cancer and to identify protein expressions that could account for mechanism of action. We treated lactating Sprague Dawley rats orally with 25 and 250 µg BPA/kg body weight or an equivalent volume of sesame oil on days 2-20. For tumorigenesis studies, 50 day old female offspring were gavaged with 30 mg dimethylbenz[a]anthracene (DMBA)/kg body weight to initiate and promote mammary cancer. Prepubertal BPA treatment resulted in a dose-dependent increase in tumor multiplicity and decreased tumor latency. Proteomic studies using 2D-PAGE and mass spectrometry determined that glucose regulated protein 78 (GRP78) was down-regulated and RAD51 homolog 2, a protein that plays a role in recombinational repair of DNA double-strand breaks, was up-regulated in mammary glands of 21 day old BPA treated rats suggesting that these two proteins respond to oxidative DNA damage of BPA. While GRP78 continues to be down-regulated at day 50, RAD51 is now down-regulated, suggesting that at time of DMBA exposure at day 50, these animals are more susceptible for chemically-induced mammary cancer. These results demonstrate that proteomic studies can provide complementary information to understanding mechanisms of action of how an endocrine disruptor (BPA) can predispose for mammary cancer. Ongoing studies are centered on using these technologies for identifying genomic and proteomic biomarkers of exposure in buccal swabs and blood of pubertal girls exposed to endocrine disruptors. Complimentary studies are being carried out in buccal swabs, blood and mammary tissues of rats exposed to BPA, phthalates and isoflavones. It is our goal to identify both genomic and proteomic biomarkers of biological responses to exposure. (Supported by Breast Cancer and Environment Research Centers grant UO1 NIEHS/NCI 12771).

Talking With Our Study Families: What, When and How to Report Study Findings

Ann Hernick, Cincinnati BCERC COTC

As the Breast Cancer and the Environment Research Centers (BCERC) begin their fifth year of transdisciplinary research, preliminary results from the epidemiology study of young girls are in. Data have been analyzed on urine biomarkers, pubertal trends and serum lipids, to name a few. While some of the results follow trends that were expected, other outcomes have been surprising. The purpose of this presentation is to present our experiences with reporting results to study families.

Center investigators and advocates have grappled with the question of what obligations we have for reporting results. There are cogent arguments for and against reporting results, particularly unexpected results. There is a plan in place to deal with clinically significant results for an individual study participant. Parents are contacted either by phone or in person to report clinical findings such as elevated lipid levels, or high blood pressure. Sometimes referrals are made as in the case of unusual psycho-social evaluations. However, we had no plan in place to report trends that we see in a community. Do we have a responsibility to report results for which we do not know their significance, cause or solution?

Community advocates argued for reporting of all results in a timely fashion. Working with researchers and clinicians, we developed procedures for reporting to study families. Our report went “beyond biomarkers”. We gave study families a thorough account of data collected in the study to date. Parental culture, language, and educational levels were considered in the development of our presentations. The response from parents was encouraging and evaluation results will be shared in this presentation. We view this reporting as an ongoing educational process and an opportunity to dialog with families as we learn from each other.
Our first obligation was to inform study families. Afterwards there was a succession of groups that also needed to be informed: public health officials, academic and clinical institutions associated with the Cincinnati BCERC and community advocates. These each required a different set of communication methods.

We are learning much from this experience. There are few models from which we could develop our communication plan. Through the transdiscliplinary nature of this research, we have had the opportunity to draw on a wide range of expertise present within other Centers and within the Working Group. It has been a team approach from both within our Center and within the network of the BCERCs.

Each BCERC will need to develop their own unique communication plan that reflects the social and cultural makeup of their study cohort in order to effectively deal with future results, both expected and unexpected..

In the last 10 to 15 years, new research paradigms have begun to evolve. Within the breast cancer advocacy community, involvement of the at risk community is a growing theme. Community advocates will continue to pressure and demand that information be shared with interested parties at the earliest possible time. As we look to the prevention of disease, research paradigms must evolve.