Variations on a Common Theme: Progesterone Regulation of Normal Mammary Gland Development in Humans, Rats and Mice

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Progesterone (P) plus estrogen (E) regulate development of the normal mammary gland. Lifetime exposure to endogenous P (early menarche, late menopause), and exogenous P in contraceptives and in menopausal hormone therapies, increases breast cancer risk. To understand the role of P in normal gland development and in the etiology of breast cancer, we have analyzed mechanism(s) of P-regulated cell proliferation in mouse and rat models. The mouse and rat differ in that the mouse only develops ducts before pregnancy, while the rat develops both ducts and lobules prior to pregnancy. The rat pattern of development is more similar to that of the human. This is due to pre-pregnancy expression of progesterone receptor B (PRB) in the rat, but not in the mouse. Additionally, in the rat there is a high percentage of cells expressing both progesterone receptor A (PRA) and PRB. This is also the case in the human breast, but occurs rarely in the mouse. We found that PRA+ cells in the mouse do not proliferate in response to P treatment, but regulate factors (RANKL, Id2, C/EBPβ) that cause cells lacking PR (PR-) to proliferate. Comparing two mouse strains, we found that C57BL/6 mice are insensitive to P by itself and require the addition of E for glandular development, while Balb/c mice are highly sensitive to P by itself (both at puberty and in adulthood). In the rat, PRA+PRB+ cells rarely proliferate because they produce factors (p21, p27) that block proliferation; PRA+PRB+ cells may produce factors that cause proliferation of PR- cells. Most P-induced proliferation in mice and rats occurs in PRB+ cells and PR- cells. In both the mouse and rat, the highest percentage of PRA+ cells is seen during puberty. These cells decrease with maturation and are permanently decreased after pregnancy. The decrease in PRA+ cells is thought to be a factor in reduced susceptibility to mammary cancer seen in individuals with an early first pregnancy. In rat mammary cancers, the percentage of PRA+ cells is increased above that of the normal gland. An increase in PRA+ cells is also observed in breast cancer and is associated with poor prognosis.

- The rat and human breast are similar in histoarchitecture, development, and PRA and PRB expression in normal and breast cancer cells. This makes the rat an excellent model to study the contribution of P to the pubertal window of susceptibility and to the etiology of breast cancer.
- The differences in P-induced regulation between the two mouse strains and similarities with regard to E or P sensitivity between the rat and the C57BL/6 mouse underscore the complexity and variations in P and E responses that are likely to be reflected in the human population.
- Despite differences between the mouse and rat, the commonality of PRA loss from puberty through pregnancy suggests a common mechanism for the protective effect of early pregnancy against breast cancer risk in the mouse, rat and human.

TGFβ1 acts as a mammary tumor promoter in the irradiated host

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Current paradigms for cancer initiation and progression generally focus on epithelial cell mutations, although it is increasingly clear that the development of cancer is highly intertwined with microenvironmental factors. We have shown that ionizing radiation induces persistent phenotypic changes in the mouse mammary stroma that are in part mediated by activation of transforming growth factor β1 (TGFβ1). We developed a stromal-epithelial mammary chimera model to determine whether the irradiated host affects mammary tumor development. This model takes advantage of the fact that the mammary epithelium can be removed from prepubertal mammary glands and the parenchyma-free stroma can then serve as the recipient of transplanted mammary epithelium. We transplanted *TP53* null mammary tissue, which recapitulate important features of human breast cancer, e.g. long latency, ductal carcinoma in situ lesions, estrogen receptor positivity. To examine whether TGFβ1 contributes to tumor promotion by host irradiation, we transplanted syngeneic *TP53* null mouse mammary fragments to Balb/c wild type (WT) and Balb/c *Tgfβ1* heterozygote (HT) mice. Inquinal glands of hosts were cleared of epithelium at 3 weeks of age and the mice irradiated whole body with 0.1, 0.5, and 1 Gy ^{60}Co γ-radiation at 12 weeks of age. p53 null mammary tissue from 10-12 week old donors was transplanted three days later. In WT hosts, more *TP53* null mammary transplants developed carcinomas at approximately a year of age compared to *Tgfβ1* HT hosts. The mean latency increased in HT hosts. Histopathological analysis revealed a pronounced difference between tumors from WT and HT hosts. WT hosts were predominantly adenocarcinomas, while HT hosts were mostly spindle cell carcinomas. These data suggest that host genotype is an important determinant of cancer progression. Tumor frequency increased to 100% in nearly all irradiated WT hosts, although it did not affect latency or tumor histology. Importantly, the effect of host irradiation on tumor frequency was ablated in *Tgfβ1* HT hosts. These data support the hypothesis that the action of radiation as a carcinogen is promoted via microenvironmental effects and that this is in large part mediated through TGFβ1.

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Puberty as a Window of Susceptibility to Environmental Exposures

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This presentation will discuss why puberty may be a critical window for the influence of environmental effects on breast cancer. The long latency of breast cancer encompasses a series of adverse genetic and other biologic changes that accumulate during the process of tumorigenesis. Some of these changes may arise during windows of susceptibility that correspond to reproductive milestones known to be associated with breast cancer risk, including birth, adolescence, and pregnancy. Racial/ethnic differences in incidence and in age at menarche suggest that puberty may be a window of risk, particularly for early-onset breast cancer, as early menarche increases risk of breast cancer. Because of variation in breast cancer incidence worldwide as well as by race/ethnicity, environmental factors are thought to elevate risk. Environmental agents can cause early development in animals as well as breast cancer. However, investigations of specific exposures with regard to breast cancer diagnosis have been inconsistent. The authors will examine the evidence for endocrine disruption by environmental exposures during different windows of susceptibility. These contaminants represent pesticides, plasticizers, cosmetic and detergent additives, and air pollutants which have different mechanisms of action relevant to breast development and carcinogenesis. Before and during puberty, these risk factors may perturb the innate endogenous hormonal system by acting in concert with the hormonal and oxidative milieu. Therefore, environmental risks must be evaluated within the context of existing knowledge about innate effectors of early and late breast development. These factors will be described, including obesity, endogenous steroid hormones, diet, physical activity and genetic susceptibility.

Unlocking the Laboratory: Introducing Breast Cancer Advocates to Bench-Top Research

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Ann Fonfa, Annie Appleseed Project and Fox Chase Cancer Center BCERC

The RFA for the Breast Cancer and the Environment Research Centers (BCERC) specified that breast cancer and environmental advocates be engaged throughout the program phases of study design, research conduct and communication of research findings. The Community Outreach and Translation Cores (COTC) foster the involvement of advocates as partners in the BCERC's research and public education objectives. A lack of knowledge about how and where laboratory research studies are conducted has hampered the involvement of community advocates in the biology studies being pursued at the Centers. Without some familiarity with how research using animal models is conducted, advocates have no framework to understand the research findings being presented or the future studies being considered. In order to educate community advocates about laboratory research methods, three different programs have been developed at three of the four BCERCs. At each center, researchers expressed interest in working with their respective COTCs and advocate partners in opening their laboratories for tours, lectures and hands-on exercises. At the University of California-San Francisco BCERC, Zero Breast Cancer as the COTC lead agency worked with a biology researcher in the development of a DVD called *Of Mice and Women: Modeling Breast Cancer and the Environment* with a companion booklet that includes take home points and a glossary of scientific terms. The BCERC at the Fox Chase Comprehensive Cancer Center created A Day in the Life of the Beast Cancer Research Laboratory, where advocates shadow researchers throughout the day and talk one-on-one with researchers about their studies. In Cincinnati, the Breast Cancer Alliance of Greater Cincinnati worked with biology and epidemiology researchers, laboratory specialists and the COTC to develop a program called Advocate Research Training (ART), a program intended to prepare advocates for more in-depth, nationally-sponsored training programs. In this presentation, representatives of the three projects will describe their respective project rationale and objectives, recruitment of participants, scope of the topics and/or procedures addressed, instruction methods, evaluation measures and outcomes, dissemination strategies, project funding and next steps or future plans. Perspectives of the scientists involved in these programs will be presented as well.