Meeting report

AACR Special Conference: Advances in Breast Cancer Research – Genetics, Biology, and Clinical Implications, Huntington Beach, California, USA, 8–12 October 2003

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Abstract

The recent meeting 'Advances in Breast Cancer Research – Genetics, Biology, and Clinical Implications' was an American Association for Cancer Research (AACR) Special Conference in Cancer Research, for which the underwriting sponsor was the Avon Foundation. Presentations were made from prominent scientists on several relevant basic science and clinic-oriented topics, including mammary stem cells and development, steroid receptors, matrix and stromal–epithelial interactions, oncogene signaling and imaging, genetics and prevention, and molecular therapeutics. A summary of recent findings is presented here, with a particular emphasis on unpublished work.

Keywords: breast cancer, mammary gland, mammary stem cells, oncogene, tumor

Introduction

The goals of the AACR Special Conference on Advances in Breast Cancer Research, organized by Carlos Arteaga (Vanderbilt University, Nashville, TN, USA) and Lewis Chodosh (University of Pennslyvania, Philadelphia, PA, USA), were to put forward the latest discoveries relevant to mammary development, transformation, breast cancer progression, and promising avenues of treatment. Approximately 225 people attended the conference from various academic research institutions and pharmaceutical companies in the United States and across the world. In addition to presentations given by speakers in the topics detailed below, there were approximately 100 poster presentations in two evening sessions. The keynote lecture, given by Philip Leder (Harvard Medical School, Boston, MA, USA), set the tone for the conference by providing a motivational presentation beginning with the impact of breast cancer on our society. He provided an overview of where the field stands, with special attention given to the utility of mouse models to address factors that may cooperate in breast tumorigenesis. In the second part of the talk, Dr Leder reviewed some of the work by his laboratory involving screens to identify small molecules that selectively inhibit growth of breast tumor cells. Lastly, he left us with a challenge: to understand some of the mechanisms behind the clinical observation that pregnancy before the age of 19 lowers one's risk of developing breast cancer. Is there a safe way to mimic pregnancy with hormonal treatment, for example, to reduce risk of breast cancer later in life? To test this, we will have to develop better models to study the phenomenon; the most popular promoters used to drive mammary-specific expression of transgenes (i.e. mouse mammary tumor virus [MMTV]) are highly responsive to hormones.

Mammary stem cells and development

The existence of mammary stem and progenitor cells has been established by a number of groups on the basis of

AACR = American Association for Cancer Research; *AIB-1* = amplified in breast cancer-1; *BRCA1/2* = breast cancer gene 1/2; CRE = cAMP response element; CREB = cyclic AMP response element binding protein; ER = estrogen receptor; ERE = estrogen response element; FGF = fibroblast growth factor; HER2 = human c-ErbB2; MAA = methoxyacetic acid; MMTV = mouse mammary tumor virus; NFκB = nuclear factor κB; PAK = p21-activated kinase; PR = progesterone receptor; PR_B = progesterone receptor, B isoform; PyMT = polyomavirus middle T (antigen); Smad = human homologue of Sma and MAD proteins; SRC = steroid receptor coactivator; TGFβ = transforming growth factor β; VEGF = vascular endothelial growth factor.

morphology (as viewed by electron microscopy), function (by serial transplantation of mammary glands or mammary epithelial cells at limiting dilutions), and as distinct, transplantable populations in the mammary gland (by the isolation of cells with flow cytometry as either a 'side population' on the basis of their ability to exclude Hoechst dye, or by expression of cell-surface markers such as Sca-1). Gilbert Smith (National Cancer Institute, Bethesda, MD, USA) reviewed his early work on the characterization of different cell populations in the mammary gland by electron microscopy and limited-dilution transplantations. He also described experiments defining the presence of bipotential progenitor cells in pregnant and lactating glands that, even after involution, can contribute to the gland in the next pregnancy. Jeffrey Rosen (Baylor College of Medicine, Houston, TX, USA) expanded on his laboratory's characterization of mammary epithelial cell lineages in the normal gland and in breast cancer. Dr Rosen described developmental data supporting a paracrine model of signaling to regulate proliferation in the mammary gland: cells expressing progesterone receptor (PR^+ cells) (which are not themselves proliferating) may secrete factors such as Wnt that can induce neighboring cells to proliferate. He noted that in ductal carcinoma *in situ*, PR^+ cells are proliferating, indicating a possible disruption of the paracrine signaling pathway during tumor development. Dr Rosen suggested that, in light of recent data implicating Wnt signaling in expansion of a progenitor-like tumor, progenitor cells may be susceptible to transformation. This could account for some of the genetic and clinical heterogeneity in breast cancer, as well as explain some recurrences after treatment has eradicated the tumor mass (a long-standing idea that is becoming more compelling in light of recent evidence).

John Wysolmerski (Yale University School of Medicine, New Haven, CT, USA) presented data regarding the role of Wnt signaling in embryonic mammary gland development. Using TOPGAL (Wnt-responsive) reporter mice, Dr Wysolmerski showed that Wnt activity increases during mammary bud formation and then decreases until the first wave of branching morphogenesis. Although several Wnt genes are expressed in mammary placodes, exogenous Wnt-3a expression in embryo cultures is sufficient to cause premature formation of placodes. Further, tetracycline-regulated expression of the Wnt inhibitor dickkopf1 (Dkk1) inhibits placode formation and the production of placode markers such as β-catenin, Tbx3, and Wnt-10b. Hence it appears that *wnt*, which is a proven oncogene in the mammary gland, not only plays a pivotal role in mammary gland development, but also may contribute to maintaining a progenitor-like state in tumors.

Steroid receptors

Orla Conneely (Baylor College of Medicine) echoed Philip Leder's sentiments about the importance of understanding why early pregnancy is protective for breast cancer. Her work has previously shown that PR_B (progesterone receptor, B isoform) is required for normal proliferation and survival of mammary epithelium, and that this is at least in part due to production of the receptor activator of nuclear factor κB (NFκB) ligand (RANKL) in PR^+ cells. This ligand, in turn, may act on neighboring cells to induce proliferation downstream of NFκB-mediated expression of cyclin D1. This also supports the notion that misexpression of PR may disrupt paracrine control of proliferation. In further support of this idea, the expression of PR in proliferating cells after carcinogenesis is prevented by early exposure to estrogen and progesterone.

Myles Brown (Dana-Farber Cancer Institute, Boston, MA, USA) spoke about modulators of steroid receptor action, and anti-estrogen therapies in particular. Selective estrogen receptor modulators, such as tamoxifen and raloxifene, generally function in mammary tissue by binding to estrogen receptor (ER) and recruiting corepressors to target promoters. Dr Brown's group is addressing whether aberrant coactivator expression or recruitment could account for resistance to tamoxifen in some breast cancers. Chromatin immunoprecipitation assays showed that while both tamoxifen and raloxifene recruit ER to target promoters, tamoxifen treatment led to the recruitment of several coactivators, including SRC (steroid receptor coactivator), AIB-1 (amplified in breast cancer-1), and cyclic AMP response element binding protein (CREB). Raloxifene, on the other hand, only caused recruitment of corepressors. Overexpression of AIB-1 in the mammary gland resulted in a larger gland, increased proliferation, precocious lobular differentiation, delayed involution, and decreased apoptosis. The mice formed mammary tumors with a latency of 12–18 months; however, reproductive history had no effect on tumor formation or latency. In a related presentation, Donald McDonnell (Duke University Medical Center, Durham, NC, USA) reported that the environmental toxicant methoxyacetic acid (MAA), which is found in paints, varnishes, and fuels, enhances tamoxifen-induced ER-mediated transcriptional activity. These data are relevant because exposure to MAA increases the risk for breast cancer in humans. MAA stimulates the MAPK (mitogen-activated protein kinase) pathway, which was also implicated in ER function by Ellis Levin (Long Beach VA Medical Center, Long Beach, CA, USA), who supported the notion that membrane-bound ER may signal through this pathway to activate nuclear ER.

The function of ER in proliferation was addressed by Peter Kushner (University of California, San Francisco, CA, USA). ERα can activate two types of target genes: it can act as a direct activator of promoters that contain estrogen response element (ERE) sites (example targets include prolactin and PR), and it can act as a coactivator

for Jun/ATF at promoters that contain cAMP response element (CRE) sites (of which cyclin D1 is a target). Dr Kushner presented elegant work with a mutant of ERα, K206A, which is a superactivator at promoters containing activator protein 1 (AP1) or CRE promoters but retains normal activation at ERE-containing promoters. Mice that overexpress ERαK206A under the MMTV promoter display exaggerated mammary gland development and increased lobular development upon stimulation with estrogen. Expression of ERαK206A under either MMTV or keratin-14 promoters resulted in hyperplasia of the mammary gland; neither MMTV-driven nor keratin-14-driven wild-type ER had this phenotype. A low incidence of mammary tumors was also reported in mice expressing ERαK206A.

Matrix and stromal–epithelial interactions

To start the session, David Cheresh (The Scripps Institute, La Jolla, CA, USA) reviewed some of his recently published work, which revealed Raf1 as a common target of vascular endothelial growth factor (VEGF)- and fibroblast growth factor (FGF)-induced survival signals in angiogenesis. The specificity of these pathways is derived from phosphorylation of Raf1 on adjacent sites; VEGF-dependent phosphorylation is dependent on p21-activated kinase 1 (PAK1), and FGF-dependent phosphorylation is dependent on Src kinase. Delivery of mutant Raf1-containing nanoparticles caused apoptosis in tumor-associated vessels as well as death in the surrounding tumor; this is attributed to bystander effect. The role of PAK in cell migration was addressed by Rudolph Juliano (University of North Carolina, Chapel Hill, NC, USA). Dr Juliano presented data demonstrating that nischarin, a recently described protein that interacts with integrins and inhibits cell migration, binds to and inhibits PAK. He proposed that upon binding of extracellular matrix, nischarin disassociates from the integrin complex, allowing cell migration via activation of Rac and PAK.

Valerie Weaver (University of Pennsylvania) presented exciting new data regarding the ligation of β integrins in response to the rigidity of the microenvironment. In three-dimensional cultures consisting of reconstituted basement membrane, cells are in a more compliant microenvironment than when in two-dimensional culture. Dr Weaver showed reduced activation of β1 integrin pathways in three-dimensional cultures, which she found to increase as the tension of the surrounding extracellular matrix was experimentally increased. These data not only emphasize the importance of the culture microenvironment in interpreting experimental studies, but also have implications for altered integrin signaling in tumors, which have higher tension due to increased collagen deposition.

Harold Moses (Vanderbilt-Ingram Cancer Center, Nashville, TN, USA) addressed the role of transforming growth factor β (TGFβ) signaling in enhancing tumor progression and metastasis of several mouse mammary tumor models. Dr Moses presented elegant new data involving deletion of the type II TGFβ receptor (βIIR) in fibroblasts. When fibroblasts lacking this receptor were mixed with a tumorigenic cell line expressing the polyomavirus middle T antigen (PyMT) and grafted into renal capsules, the resulting tumors were approximately 2.5 times as large as those reconstituted with wild-type fibroblasts. These data support a positive role for TGFβ signaling in tumors via a paracrine mechanism.

Oncogene signaling and imaging

As a result of continuing development of new tools and model systems in which to study breast cancer, there has been significant progress in understanding mechanisms of tumorigenesis, cell invasion, and metastasis. Joan Brugge (Harvard Medical School) presented work from her laboratory pertaining to the reconstruction of tumor phenotypes with MCF10A cells in three-dimensional cultures using retroviral delivery of oncogenes. Using this powerful method, they have been able to identify genes that induce hyperplasia (for example, the genes for ErbB2, colony stimulating factor 1, colony stimulating factor receptor, insulin-like growth factor 1, insulin-like growth factor receptor, c-Met, and hepatocyte growth factor), a more intermediate phenotype (for example, Wnt-1, intracellular notch, activated Akt), and combinations of genes whose proteins induce an invasive phenotype (for example, ErbB2 + TGFβ). Her laboratory is currently making progress on the mechanisms involved in the various tumor phenotypes.

William Muller (McGill University, Montreal, Canada) also addressed mechanisms of tumor formation and invasion. Using elegant mouse genetics, he demonstrated that some target(s) of phosphatidylinositol 3-kinase, other than Akt, are required for pulmonary metastasis of MMTV–PyMT tumors. In another system, he showed that β1 integrin is critical for progression of MMTV–PyMT tumorigenesis. Lewis Chodosh (University of Pennsylvania) and his group are utilizing mammary-specific, doxycycline-inducible mouse models to characterize not only the fine points of tumor progression, but also whether tumors remain dependent on the initiating event. As he has shown in recent publications, several factors can influence tumor regression and recurrence: mutations in *K-ras* can render Myc-induced tumors resistant to reversal, and loss of one allele of p53 can contribute to recurrence of Wnt-induced tumors. Dr Chodosh also presented interesting data suggesting that, once regressed, residual neoplastic disease can remain dormant for some time; reinduction of the oncogene leads to tumor formation at a much faster rate than was seen with formation of the initial tumor. The theme of dissecting molecular pathways that contribute to tumorigenesis was continued by Peter Sicinski (Dana-

Farber Cancer Institute), who presented recently published work from his laboratory on the phenotype of cyclin E1/E2 double-knockout animals. Future work on the requirement for these cyclins in progression of mammary tumors is greatly anticipated.

Lastly, there was some discussion on methods to image tumor progression. Ronald Blasberg (Memorial Sloan-Kettering Cancer Center, New York, NY, USA) gave an overview of methods of noninvasive imaging, with a particular emphasis on reporter imaging. His laboratory has used various imaging systems to dissect several endogenous molecular pathways, including activation of hypoxia-inducible factor α in xenografts using a hypoxia-responsive promoter that drives expression of green fluorescent protein.

Genetics and prevention

Thea Tlsty (University of California, San Francisco) started off this session with a comprehensive talk covering early genetic and epigenetic events in human breast cancer. Using an *in vitro* model system to identify human mammary epithelial cell 'variants' that escape normal proliferation barriers, Dr Tlsty's group has identified a number of early events in breast cancer, including chromosomal abnormalities such as telomeric association, abnormal centrosome numbers, and hypermethylation of the p16 promoter. Importantly, overexpression of wild-type p16 in these variants can induce a senescent-like phenotype, and 'knocking down' p16 expression with siRNA (small interfering RNA) in normal cells leads to abnormal centrosome numbers through a failure to couple centrosome duplication with the cell cycle. These data support a critical role for p16 in genomic stability of breast epithelial cells.

Michael Kastan (Saint Jude Children's Research Hospital, Memphis, TN, USA) reminded us that cancer susceptibility is often linked to responses to DNA damage. His laboratory has found that breaks in DNA, which cause relaxation of tightly wound chromatin, may be an important signal for activation of ataxia-telangiectasia-mutated kinase to initiate the cellular response to DNA damage. The topic of DNA repair was continued by Alan D'Andrea (Dana-Farber Cancer Institute), who reviewed details regarding cooperation between the Fanconi anemia DNA damage repair complexes and BRCA1/2. He also showed that ATR (ataxia-telangiectasia and Rad3-related protein), mutations of which lead to Seckel syndrome, may be a sensor kinase to turn on the Fanconi/BRCA DNA damage pathway. To wrap up the session, David Livingston (Dana-Farber Cancer Institute) demonstrated specific localization of BRCA1 at sites of DNA damage. This localization requires the C-terminus of the BRCA1 protein, and proper localization of BRCA2 is dependent on BRCA1. Dr Livingston also presented data building on his previous report of interaction of BRCA1 with RNA of Xi-specific transcripts on the inactive X chromosome. He showed intriguing data that the presence of RNA could also affect colocalization of BRCA1 with pericentromeric heterochromatin after DNA damage. These data lead one to speculate whether interaction with RNA might contribute to the DNA repair function of BRCA1.

Molecular therapeutics

Carlos Arteaga (Vanderbilt University) presented data from his laboratory addressing mechanisms of the putative switch of TGFβ from tumor suppressor to tumor promoter. He showed that very low levels of TGFβ receptor activation induce effects independent of Smad (the human homologue of Sma and MAD proteins), including activation of Akt and Erk kinases. In vivo, TGFβ activation in MMTV-*neu* mice results in less differentiated, more invasive tumors. Treatment of tumor explants in matrigel with TGFβ inhibitors such as LY580276 reverts the invasive behavior and induces apoptosis. Together, these data provide evidence of the potential efficacy of TGFβ inhibitors in disrupting both tumor maintenance and metastasis.

Powel Brown (Baylor College of Medicine) addressed the need for therapeutics for ER^- breast cancer. Dr Brown presented data showing that ZD1839 (Iressa), a selective inhibitor of epidermal growth factor receptor–tyrosine kinase, can reduce proliferation of xenografts of human ductal carcinoma *in situ* in mice. Additionally, both Iressa and retinoids can suppress development of ER^- (MMTV-*neu*) tumors in mice when administered chronically. These data, and others, have led to a current clinical trial on the efficacy of the retinoid LGD1069, which is selective for retinoid-X receptor, in preventing the disease in women at high risk.

C Kent Osborne (Baylor College of Medicine) provided an overview of the latest clinical findings that aromatase inhibitors are beneficial after treatment with selective estrogen receptor modulators in ER^+ breast cancer. He expanded on mechanisms for hormonal resistance and his recent data suggesting that resistance to tamixofen correlates with high levels of both AIB1 and HER2 (human c-ErbB2) overexpression. In xenograft studies, inhibition of both ER and growth factor signaling (with tamixofen and Iressa) is more effective than inhibiting either pathway alone.

Lastly, Keith Knutson (University of Washington, Seattle, WA, USA) showed that immunization of animals with Neu intracellular domain peptides before exposure to Neu-expressing tumors acutely reduced tumor growth. Stage III and IV clinical trials, in which patients were vaccinated once per month for 6 months with HER2/Neu vaccines, showed no toxicity. Other results from the study showed that the majority of patients developed 'epitope spread-

ing', or a broadening of the immune response, and immunity persisted for at least 1 year in half of the patients.

Barriers and motivators to breast cancer screening

Despite constant advances in our understanding of the molecular basis of breast cancer and the generation of more effective therapeutics, a critical component of the outcome of the disease is early detection. Marydale DeBor (lawyer and senior consultant for the Avon Foundation) reported that the Avon Foundation is the largest private contributor to breast cancer research; it has raised more than $250 million over the past 10 years for basic and translational research, as well as issues directly pertaining to patient care. Barbara Cicatelli (Avon Foundation Breast Care Fund, New York, NY, USA) presented an enlightening study of more than 100,000 women to determine the major barriers to breast cancer screening, from both patients' and caregivers' perspectives. Interestingly, while patients most often described barriers such as personal belief (the view that one's health is in God's hands) and a lack of perceived risk, heath care providers thought that the major barriers were based on fear or were financial. Indeed, finance was a barrier described by both patients and caregivers. Motivators for screening were similar between the groups, and included a sense of self-empowerment and knowledge that treatment is available. This study should provide a foundation for improved breast health education and screening for earlier detection of the disease.

Conclusions

Ultimate progress in the understanding and treatment of breast cancer arises from basic and translational research in many disciplines. Recent findings in progenitor cell function and mammary development will contribute insight to the consequences of the aberrant re-activation of developmental programs in many tumors. Research on basic steroid hormone biology will ultimately add to the design of hormone-based therapeutics. Understanding stromal–epithelial interactions, and the behavior of particular oncogenes and how they interact with various genetic anomalies in breast cancer, are also promising avenues to molecular therapy. This conference provided a comprehensive collection of all of these approaches and should foster fresh thinking and collaborations to achieve the common goal.

Competing interests

None declared

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