Meeting report

Department of Defense Era of Hope Meeting, Orlando, Florida, USA, 25–28 September 2002

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Abstract

The *Era of Hope* meeting addressed with a multidisciplinary approach the most critical issues in breast carcinogenesis. The issues that we summarize here include: a) the use of rodent models for the study of mammary gland development and breast tumorigenesis; b) the effects of stroma on mammary epithelial differentiation and malignant transformation; c) a further characterization of the interactions between steroid and growth factor receptors; d) the improvement of technologies for early detection of breast tumors and the establishment of their progression; and e) the development of vaccines as potential new therapies against specific tumor markers.

Keywords: animal models, breast cancer, imaging, mammary gland development, stromal-epithelial interactions

Introduction

The 10th anniversary of the 'Era of Hope' meeting sponsored by the US Department of Defense (DOD) Breast Cancer Research Program (BCRP) was held in Orlando, Florida, USA, during 25–28 September 2002. Basic scientists, clinicians, policymakers, breast cancer survivors and advocates attended the meeting to learn about advancements in breast cancer research. The mix of individuals with such diverse backgrounds provided a unique atmosphere to this meeting. Each day of the meeting began with educational sessions that covered topics from breast pathology to epidemiology to technologies for early detection. Three innovator sessions were held, one on each day, to present innovative ideas and results that encouraged the delegates to think creatively about new strategies for breast cancer. These sessions ranged from the cell biology of breast cancer and the role of polarity and epigenetics, given by Mina Bissell (Lawrence Berkeley National Laboratory, California, USA) [1,2], through tumor immunology, presented by Mary Lenora Disis (University of Washington in Seattle, Seattle, Washington, USA) [3,4], to creative approaches in problem solving, by Dean Kramer (Segway, LLC, Manchester, New Hampshire, USA).

An unusual aspect of the conference was interactive sessions conducted in an open-forum format so that members of the audience and panel members could freely discuss critical issues to be addressed in the future in breast cancer research. In addition to keynote and plenary sessions, there were several focused symposia and poster sessions that highlighted the research of the recipients of DOD-BCRP grants. An inspirational feature of the meeting was that scientists and breast cancer advocates co-chaired the symposia. This reinforced a sense of collaboration between the women who have had breast cancer and the researchers, a collaboration that should help to define the important areas that need to be explored and addressed in breast cancer research. Owing to the extensive amount of research presented, the following report will include summaries of only a representative sampling of the excellent science that was presented.

Rodent models of mammary gland development, tumorigenesis and metastasis

An early educational session presented by Jeffrey M Rosen (Baylor College of Medicine, Houston, Texas, USA) focused on the importance of studying normal mammary

BCRP = Breast Cancer Research Program; DCIS = ductal carcinoma *in situ*; DOD = US Department of Defense; ER = estrogen receptor; MMP = matrix metalloproteinase; MRI = magnetic resonance imaging; TGFβ = transforming growth factor β; THC = *R*,*R*-5,11-*cis*-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol; VEGF = vascular endothelial growth factor.

gland development for understanding how breast cancer arises. The same molecular events controlling normal development, when deregulated, result in cancer. In this overview, Rosen referred to cell polarity, steroid receptors, growth factors and p53 as crucial factors in mammary gland development and tumor progression. Quiescent mammary stem cells with significant regenerative capacity might have a role in tumorigenesis. In this regard, his recent publication [5] showed that cells positive for stem cell antigen-1 represent an enriched population of progenitor cells and have increased regenerative potential when transplanted into cleared fat pads. In addition, several presentations addressed signaling molecules involved in branching morphogenesis and mammary tumorigenesis. For example, Eran Andrechek (Department of Biology, McMaster University Hamilton, Ontario, Canada) showed that conditional activation of *neu* under the endogenous promoter resulted in accelerated lobulo-alveolar development and altered branching morphogenesis of the mammary gland.

An interactive session brought forward various concerns about the sufficiency of rodent models for the study of human mammary tumorigenesis. One concern emerged from these discussions, namely that current mouse models are inadequate for the study of metastatic breast cancer because mouse mammary tumors do not typically metastasize; neither do the tumors resemble human pathology. However, one mouse model showing lung metastasis and tumor recurrence was presented by Susan Moody (University of Pennsylvania, Philadelphia, Pennsylvania, USA) using transgenic mice that overexpressed *neu* under the tetracycline-inducible promoter. Graeme Hodgson and collaborators (University of California, San Francisco, California, USA) have used comparative genomic hybridization to identify specific abnormalities of genomic copy number leading to metastasis with the use of two transgenic mouse models, erbB2 and polyomavirus middle T antigen. These mice develop mammary tumors that often metastasize to the lung. Marcia Noble (Georgetown University, Washington DC, USA) presented a bitransgenic mouse model in which overexpression of c-*myc* and vascular endothelial growth factor (VEGF) showed increased angiogenesis and metastasis. As an alternative to mouse models, transgenic rat models are being developed by Michael Gould (University of Wisconsin, Madison, Wisconsin, USA) because they display mammary tumor morphology with more resemblance to human hormone-responsive breast tumors.

Angiogenesis and proteinases

Understanding mechanisms involved in angiogenesis and finding therapies to inhibit angiogenesis are important topics in breast cancer research. In a plenary session, Renata Pasqualini (University of Texas MD Anderson Cancer Center, Houston, Texas, USA) presented an approach to determine 'molecular addresses' in blood vessels with an *in vivo* phage display. Tumor-homing peptides can be designed to deliver therapeutic or imaging agents directly to the tumor (reviewed in [6]). In addition, several posters presented data characterizing the role of VEGF, its receptors, and signaling pathways to uncover novel ways of disrupting angiogenesis in breast tumor progression. Proteinases have an integral role in angiogenesis, because for new blood vessels to form, components of the extracellular matrix must be degraded and remodeled. There were several posters characterizing the role of serine proteinases and their inhibitors in breast cancer. Additionally, there were several presentations on metalloproteinases, in particular matrix metalloproteinases (MMPs). Data were presented by the laboratory of Constance Brinckerhoff (Dartmouth Medical School, Hanover, New Hampshire, USA) that characterized the effect of a single nucleotide polymorphism occurring in the MMP-1 promoter. Other posters, such as those presented by the laboratory of Rafael Fridman (Wayne State University, Detroit, Michigan, USA) examined the regulation of MMP activation [7].

Stromal influences on mammary epithelium

Stromal effects on mammary epithelial differentiation and tumor progression were the topic of several presentations. One of the factors involved in epithelial–stromal communication is transforming growth factor β (TGFβ). Michael R Crowley and Rose Serra (University of Cincinnati, Cincinnati, Ohio, USA) developed a transgenic mouse model that expressed a zinc-inducible kinase-defective dominant-negative TGFβ type II receptor in the mammary stroma. These mice displayed increased lateral branching, suggesting that TGFβ signaling in the stroma has important effects on epithelial morphogenesis. Maria Jose Pajares (Lawrence Berkeley National Laboratory, Berkeley, California, USA) showed that ionizing radiation at a low dose caused reduced mammary epithelial apoptosis and p53 activation in a TGFβ1 heterozygous mouse model in comparison with wild-type mice. This suggests that TGFβ1 activation in response to radiation damage can alter cell fate decisions.

A symposium of particular interest was focused on the effect of stromal–epithelial interactions in breast cancer. In particular, Hema Parmar (University of California, San Francisco, California, USA) presented an interesting model in which human mammary ductal organoids were co-cultured with human fibroblasts in a collagen type I gel that was grafted under the renal capsule of female nude mice. By means of this technique, normal human mammary morphology was recapitulated. When normal fibroblasts were replaced by mammary carcinoma-associated fibroblasts, aberrant ductal morphology resulted.

Crosstalk between steroid receptors and growth factor receptors

Some of the new approaches regarding steroid hormones as therapeutic targets were presented. Geoffrey L Greene

(University of California, San Francisco, California, USA) described the use of the synthetic compound (*R*,*R*)-5,11-*cis*-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (THC) as a selective estrogen agonist when bound to estrogen receptor (ER)α and as an antagonist when bound to ERβ. The mechanism of ligand discrimination by THC has recently been published [8]. In contrast, Jennifer Richer (University of Colorado, Denver, Colorado, USA) proposed that an inappropriate sensitivity to estrogens and progestins could be due to the overexpression of members of the Kruppel-like zinc-finger and winged-helix domain families of transcription factors. These factors were shown to be upregulated by progesterone in T47D human breast cancer cells.

In an interactive session, alternative treatments and combinatorial therapies targeting growth factor receptor pathways to prevent resistance to anti-hormonal therapy were advocated. Several other presentations were also focused on the crosstalk between steroids and peptide growth factors. Richard Pietras (University of California, Los Angeles, California, USA) described the membrane-associated ER that localized to caveola-related structures, where it interacts with HER-2/neu growth factor receptor. This interaction produces an acute activation of mitogen-activated protein kinase and Akt [9], which promotes intracellular signaling for estrogen-mediated proliferation that could be a new target for antitumor therapy. In contrast, c-Src tyrosine kinase was proposed by William J Muller (McMaster University, Hamilton, Ontario, Canada) to mediate ER phosphorylation and transcriptional activation, and to be required for normal mammary ductal outgrowth. In addition, the involvement of AIB1 (amplified in breast cancer-1), a steroid receptor coactivator, in integrating signals from steroid hormones and growth factors, as well as its role in hormone-dependent transcriptional activation of ER, was discussed in several presentations. These data, and the fact that AIB1 overexpression leads to altered ductal growth and breast cancer, make this co-activator a potential target for therapy and breast cancer prevention.

New technologies applied to understanding breast tumor progression

Ductal carcinoma *in situ* (DCIS) occurs in about 30% of breast tumors. However, many questioned whether DCIS is currently being overtreated. To permit better diagnosis and prediction of the progression of DCIS, improved imaging methods to determine the grade, extent and margins of the tumor are crucial. A computer-assisted three-dimensional microscopy system to model DCIS lesions, using fully sectioned tissue samples, is being developed by Carlos Ortiz de Solórzano (Lawrence Berkeley National Laboratory, Berkeley, California, USA). An improvement in magnetic resonance imaging (MRI) to provide better contrast, texture and edge delineation than conventional MRI is being developed by GS Karczmar (University of Chicago, Chicago, Illinois, USA) and by Savannah Partridge (University of California, San Francisco, California, USA).

Other novel imaging methods being developed include near-infrared spectroscopy (Hanli Liu, University of Texas Southwestern Medical Center, Dallas, Texas, USA), laser optoacoustic tomography (Alexander Oraevsky and Sergey Solomatin, University of Texas Medical Branch, Galveston, Texas, USA), and breast cancer radars and microwave imaging to contrast high water content in tumors and low water content in normal tissues (Jack Bridges, Mt Prospect, Illinois, USA). In contrast, gene expression profiling using cDNA arrays (discussed by Dennis Sgroi, Harvard Medical School, Boston, Massachusetts, USA) and proteomics (discussed by Patricia Steeg, National Cancer Institute, NIH, Bethesda, Maryland, USA) are other potential tools for determining tumor grade and progression. David Botstein (Stanford University, Palo Alto, California, USA) highlighted the use of microarray technology in the characterization of breast cancers. Results from the array data can potentially be used to determine types of therapy.

Development of vaccines against breast cancer

In several immunology sessions, data were presented in connection with the possibility of developing vaccines to increase T-cell response against tumor cells. Some groups have developed specific vaccines targeting ErbB-2/HER-2/neu, seeking to change tumor tolerance and improve antigen presentation in mouse models. AM Ercolini (The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA) has characterized a major histocompatibility complex class I epitope in a rat *neu-N* transgenic mouse model that accomplished both aims. By genetically linking IgG Fc fragments and heat shock proteins to the rat *neu* gene, Xue F Huang (Baylor College of Medicine, Houston, Texas, USA) showed enhanced antigen presentation by receptor-mediated internalization of dendritic cells. Another strategy with a gene-based approach to the induction of a specific T-cell response against Her-2/neu antigen in mice was presented by Lawrence Lachman (University of Texas, MD Anderson Cancer Center, Houston, Texas, USA) and Brian Long (University of North Carolina, Chapel Hill, North Carolina, USA).

DNA repair in breast cancer

Genetic instability is a common feature of human cancers. Thus, it is important to understand mechanisms involved in DNA repair. Mark Brenneman (University of New Mexico, Albuquerque, New Mexico, USA) and Yi-Ching Lio (Lawrence Berkeley National Laboratory, Berkeley, California, USA) found that disruption of the interaction between BRCA2 (breast cancer suppressor gene 2) and RAD51

(part of a multiprotein complex that participates in homologous recombination) significantly decreased homologous recombinational repair. Jean Latimer (University of Pittsburgh, Pittsburgh, Ohio, USA) showed that both breast tumor and adjacent non-tumor tissue manifested a deficiency of nucleotide excision repair, and that proteins with DNA helicase activity might be involved. Guo-Min Li (University of Kentucky, Lexington, Kentucky, USA) observed genetic and epigenetic alterations of two mismatch repair genes, h*MSH2* and h*MLH1*, in sporadic breast tumors with microsatellite instability.

Conclusion

A main objective of the DOD-BCRP Meeting is to bring together in a friendly and interactive environment a multidisciplinary spectrum of people with the common aim of understanding, preventing and controlling breast cancer. This format was clearly successful; this multidisciplinary interaction should continue in the future, to facilitate early detection and to improve therapies for breast cancer. Here we have presented a summary of some relevant symposia and posters presented at the conference. A complete list of abstracts can be found at http://cdmrp.army.mil/bcrp/era/default.htm.

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