Meeting report

26th Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, USA, 3–6 December 2003: update on preclinical and translational research

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Abstract

The San Antonio Breast Cancer Symposium is the largest annual meeting devoted solely to breast cancer research. The late William L McGuire's vision for this meeting was to stimulate 'translational research', many years before this term became popular. In this way, the San Antonio Breast Cancer Symposium represents a forum in which basic and clinical researchers present their research side by side. Each year sees the continued evolution of our understanding of the basic mechanisms of breast cancer initiation and progression, and the clinical application of this knowledge. Major topics of discussion at the symposium this year were the cell cycle, new evolving concepts of estrogen receptor action, breast cancer stem cells, new predictive and prognostic markers (including microarray studies), and continued exploration of the mechanisms of drug resistance. This report will summarize preclinical and translational highlights from the meeting.

Keywords: BRCA1, cell cycle, drug resistance, estrogen receptor, growth factor receptors, microarray, prognostic and predictive markers

Introduction

The 26th Annual San Antonio Breast Cancer Symposium was held in San Antonio, Texas on 3–6 December 2003. Over the past 26 years this meeting has evolved into the largest conference in the world devoted solely to breast cancer research. This year there were more than 700 abstract presentations, from 6000 attendees representing 80 countries. This not only included physicians and scientists who presented the newest information on prevention, diagnosis, and treatment of breast cancer, but also included breast cancer patient advocates. In the spirit of the late William L McGuire, who co-founded this symposium with Charles A Coltman Jr in 1978, cellular and molecular biology with translational potential was presented, and the highlights will now be discussed.

Stem cells

The meeting was opened by a plenary lecture on stem cells in normal breast development and breast cancer by Max Wicha (University of Michigan, Ann Arbor, Michigan,

USA). Wicha pointed out that many features of stem cells are also shared with breast cancer cells, including the ability to self renew and differentiate, telomerase activity, resistance to damaging agents, and anchorage-independent growth and survival (abstract P1 [1]).

Wicha has developed a new *in vitro* culture system allowing the propagation of putative stem cells from normal breast tissue [2]. In this situation, cells grow in perfect spheroids, termed mammospheres, and show the two classic features of stem cells: the ability both to self renew and to differentiate. Microarray analysis of these cells showed expression of many genes that are similar to those expressed in hemopoetic cells, neuronal cells, and embryonic stem cells. Importantly, when overexpressed in the mammary gland, many of these genes result in tumorigenesis.

Wicha went on to show that breast cancers contain putative cancer stem cells that can be selected by specific cell surface markers such as CD44 and CD24. Blockade of

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cdk = cyclin-dependent kinase; ER = estrogen receptor; PCR = polymerase chain reaction; RT = reverse transcription.

the Notch 4 ligand, which is highly expressed in normal stem cells, can inhibit tumorigenesis by the putative cancer stem cells, highlighting a possible novel target for breast cancer therapy.

The cell cycle

Cell cycle deregulation is an extremely important element in breast cancer progression, and its deregulation reflects an attractive target for therapeutic intervention. This area was given special coverage in a mini-symposium entitled 'Cell Cycle Regulators – Targets for Therapy?'.

Richard Pestell (Georgetown University, Washington, DC, USA) gave an overall introduction to the cell cycle, but stressed that our reductionist models of cyclin action can be misleading (abstract MS1-1 [1]). To this end, Pestell showed that cyclin D_1 has several novel functions that seem to be unrelated to its action in the G_0/G_1 phase of the cell cycle. In particular, migration and invasion are reduced in cyclin D_1-deficient macrophages [3]. In addition, cyclin D_1 was found to be an inhibitor of the peroxisome proliferator-activated receptor gamma, as ligands that activate this receptor cause increased adipocyte differentiation in cyclin D_1-deficient fibroblasts [4].

Khandan Keyomarsi (University of Texas MD Anderson Cancer Center, Houston, Texas, USA) emphasized that breast cancer reflects deregulation of cell cycle control and not simply increased rates of cell growth (abstract MS1-2 [1]). Keyomarsi showed that cyclin E levels have prognostic value in breast cancer, with patients having high levels of cyclin E exhibiting shorter disease-free survival [5]. Furthermore, immunoblot analysis revealed multiple truncated forms of cyclin E, which Keyomarsi has shown to be associated with increased cdk2 activity. Overexpression of these smaller truncation products in cell lines resulted in enhanced S-phase entry, and overexpression in the mammary glands of mice caused mammary tumorigenesis. The small, truncated products are a result of cleavage of cyclin E by elastase. Interestingly, a natural inhibitor of elastase exists, termed elafin, and expression of elafin is markedly reduced in breast cancer cell lines compared with normal cell lines. Keyomarsi has thus provided an attractive hypothesis whereby loss of elafin leads to enhanced cleavage of cyclin E by elastase, forming highly active truncation products.

Sandra Swain (National Cancer Institute, Bethesda, Maryland, USA) reviewed the development and clinical use of cyclin-dependent kinase (cdk) inhibitors. The first inhibitors (flavopiridol and UCN01) show high potency but are active against all of the cdks. Flavopiridol showed little activity in phase I and phase II clinical trials, and it had unacceptable toxicity when combined with chemotherapy. UCN01 showed better activity in phase I trials and is now in phase II trials. Subsequent cdk inhibitors such as

roscovitine and BMS38702 are more selective for certain cdks, but are less potent. These compounds are currently in preclinical and clinical development. The minimal clinical activity of the cdk inhibitors as single agents is similar to that seen to date with tyrosine kinase inhibitors and angiogenesis inhibitors. This activity highlights the need to have a better understanding of the basic mechanisms of signaling in these systems and to define biomarkers to select patients who are likely to respond to these inhibitors.

Prognostic markers

While studies of single factors have generally failed to provide new prognostic biomarkers with clinical utility, the hope is that new technologies evaluating many genes or their products simultaneously may provide new prognosticators. In this regard, Soon Paik (NSABP, Pittsburgh, Pennsylvania, USA) presented a multigene PCR assay that can predict recurrence in node-negative breast cancer patients (abstract 16 [1]). Paik first identified a large set of ~250 candidate genes based on previous microarray data sets and other literature, and then measured these genes in archival paraffin-embedded tissue using a multigene RT-PCR method. A subset of genes were identified that correlated with the likelihood of breast cancer recurrence, and a recurrence score based on 21 genes (including five reference genes) was developed. These 21 genes were then measured in estrogen receptor (ER)-positive node-negative patients (NSABP B-20) and showed a highly significant ability to predict disease recurrence, with the RT-PCR assay being the strongest predictor in multivariate analysis.

This abstract, however, was followed by a presentation by Francisco Esteva (University of Texas MD Anderson Cancer Center), who showed that the same multigene RT-PCR assay was not able to predict recurrence in a set of ER-positive untreated patients (abstract 17 [1]). The discordance in these studies may reflect the uniqueness of Esteva's study set (which unexpectedly showed that high nuclear grade was associated with favorable outcome) or may reflect that the training set represented patients who were all treated with hormone therapy, thus reflecting the weight assigned to ER-regulated genes in the 21-gene set. More studies will be needed to determine the utility of this prognostic multigene RT-PCR assay.

Other investigators utilized microarray technology to predict response to therapy. Els Bern (JNI, Rotterdam, The Netherlands) showed that an 81-gene set that was differentially expressed between tamoxifen-sensitive and tamoxifen-resistant tumors could be used to accurately predict response in approximately 80% of cases (abstract 26 [1]). In order to characterize response to chemotherapeutic drugs, Debu Tripathy (University of Texas Southwestern Medical Center, Dallas, Texas, USA) subjected 15 breast cancer cell lines with differential sensitivity to cisplatin,

doxorubicin, paclitaxel, and vinorelbine to microarray analysis (abstract 30 [1]). Response-associated patterns were identified, but these showed little overlap between the different drugs, indicating that different mechanisms are involved. Expanding the studies to a larger number of cell lines might result in the identification of more gene expression markers in common, at least for drugs with similar mechanisms of action.

Craig Allred (Baylor College of Medicine, Houston, Texas, USA) presented microarray analysis of the differences between ductal carcinoma *in situ* and invasive breast cancer. His studies revealed more than 1000 genes that differed in expression, and these genes allowed near-perfect diagnostic separation (96–100%) between ductal carcinoma *in situ* and invasive breast cancer (abstract 13 [1]). Genes important in this separation were involved in membrane signal transduction, the extracellular matrix, cell motility, and cell adhesion.

Chris Jones (Institute of Cancer Research, London, UK) presented an elegant array study analyzing the differences between normal luminal epithelial cells and myoepithelial cells (abstract 20 [1]). The authors identified 72 genes more highly expressed in the luminal epithelial cells, and identified 132 genes more highly expressed in the myoepithelial cells. Where antibodies were available, specific overexpression was confirmed by immunohistochemistry in breast tumor tissue arrays. Most noteworthy, they identified some proteins such as galectin-3 and osteonectin that were strong prognostic indicators.

The ER – still surprises?

As indicated by the title of this mini-symposium, 'The Estrogen Receptor – Still Surprises?', the ER keeps giving scientists new challenges and puzzles. Novel ER actions on the membrane, activity that is independent of its direct DNA binding, and its interaction with the tumor suppressor gene BRCA1 were discussed.

Ellis Levin (Long Beach Veterans Medical Administration Center, Long Beach, California, USA) indicated that the ER, via its c-terminal E domain, can interact with caveolin and reside in caveolin pits in the plasma membrane. Here the ER can interact with both growth factor and G-protein coupled pathways to provide signals termed membrane-initiated steroid signaling, which may be critical for estrogen's ability to promote survival in such tissues as bone, the heart, and the brain. In addition, Levin showed that BRCA1 can inhibit membrane-initiated steroid signaling to ERK1/2 by increasing the expression of the ERK1/2 phosphatase MKP1 (abstract MS3-1 [1]).

Data on how the ER contributes to proliferation without directly binding to DNA but instead by binding and activating the transcription factor AP-1 were presented by Peter Kushner (University of California, San Francisco, California, USA) (abstract MS3-2 [1]). Kushner highlighted a mutant ER (K206A) that shows hypersensitive ER action on AP-1 elements, but has unchanged activity on an estrogen response element. Overexpression of this mutant ER in the mammary glands of mice led to hyperplasia and mammary tumorigenesis, although overexpression of the wild-type ER also caused mammary tumors, indicating the potential oncogenic action of the ER.

Why do BRCA1 and BRCA2 mutations cause breast cancer, ovarian cancer, and prostate cancer instead of cancers in other organs? Data presented by Eliot Rosen (Georgetown University) might provide an answer (abstract MS3-3 [1]). Rosen showed that BRCA1 interacts with and represses transcriptional activity of the ER, thus inhibiting the cell's response to estrogen [6]. While details are yet to be discovered, it seems that direct binding and recruitment of repressive proteins, as well as inhibition of the coactivator p300, are involved. Most relevant, however, was the finding that BRCA mutations found in tumors fail to repress the ER, thus providing an exciting hypothesis of why mutation carriers are especially susceptible to cancer formation in hormone-responsive organs. While breast cancer in BRCA1 mutation carriers is often ER-negative, an inability of BRCA1 to inhibit ER action may explain why bilateral oophorectomy is able to reduce breast cancer incidence in these patients.

Drug resistance

Several presentations dealt with the problem of drug resistance in breast cancer. A number of these highlighted the role of tyrosine kinase receptors, in particular the epidermal growth factor receptor family, in mediating resistance to hormone therapy. Steve Johnston (Royal Marsden Hospital, London, UK) highlighted how growth factor receptors can activate the ER in a ligand-independent manner and cause antiestrogen resistance (abstract MS3-4 [1]). Johnston also noted that inhibition of this cross-talk is being tested in a number of current clinical trials using inhibitors to either growth factor receptors or their downstream signaling components.

In this regard, two preclinical studies showed that combining an epidermal growth factor receptor inhibitor (gefitinib [Iressa]; Astrazeneca, London, UK) and/or a HER-2 inhibitor (trastuzumab [Herceptin]; Genentech, San Francisco, California, USA) with antiestrogen therapy can enhance the response and delay the resistance in MCF-7 breast cancer cells engineered to overexpress HER-2 (abstracts 22 and 25 [1]). Another presentation, however, showed that MCF-7 cells that become resistant to tamoxifen and Iressa show elevated insulin-like growth factor-I receptor signaling (abstract 1011 [1]), indicating that there may be yet another pathway to resistance. This suggests that targeting of multiple receptors may be necessary.

Conclusion

The 26th San Antonio meeting continued to reflect the breadth of translational research in breast cancer. Advances in the understanding of the fundamental basics of breast cancer initiation and progression are providing unique opportunities for breast cancer therapeutic intervention. Mini-symposiums such as the one devoted to the ER, however, show that continued basic and translational research is needed to fully appreciate the mechanisms of action of targeted therapies, and hopefully to combat drug resistance.

Competing interests

None declared.

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