

Meeting report

91st Annual Meeting of the American Association for Cancer Research

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Introduction

The 91st Annual Meeting of the American Association for Cancer Research (AACR) was held at the Moscone Convention Center in San Francisco, California, USA, April 1–5, 2000. The comprehensive and multidisciplinary nature of the AACR meeting was conveyed through a collection of concurrent symposia, minisymposia and poster sessions. These standard meeting forums were further supplemented by additional educational workshops, interactive 'meet-the-expert' sessions at the beginning of each day and panel discussions approaching more general interest topics, such as the relationship of media, science and consumers. Although it was generally impossible to attend all of the sessions on a particular topic, many topics were repeated in different formats throughout the meeting to allow for attendees to get a reasonable sampling of the current trends in each field.

A number of scientists were honoured at the meeting for their outstanding contributions to cancer research. Charles Sherr (St Jude's Children's Research Hospital, Memphis, TN, USA) was awarded the Pezcoller International Cancer Research Award for his work on the mechanisms of cell growth control and neoplastic transformation. The Bruce F Cain Memorial Award was given to Axel Ullrich (Max-Planck Institute for Biochemistry, Martinsried, Germany) who has successfully translated his pioneering work on tyrosine kinase receptors, such as HER2/neu, into actual treatment strategies. Edison T Liu (National Cancer Institute, Bethesda, MD, USA) was also recognized for his work in establishing a correlation between HER2/neu overexpression and those breast cancers that have an unfavourable prognosis and high probability of

responding to doxorubicin therapy. Finally, the prestigious GHA Clowes Memorial Award was presented to Elizabeth Blackburn (University of California, San Francisco, CA, USA) for her pioneering work in the discovery of telomerase and its potential role in cancer.

Herein we outline a few of the many provocative studies discussed at the meeting. Although some of the topics discussed below are specific to the breast, others addressing global mechanisms of tumour progression are also considered because they may be appropriate paradigms for understanding and treating breast cancer in the future.

Steroid and steroid receptor function in breast cancer progression

The role of steroids and their receptors in breast cancer progression was the focus of a number of presentations.

In an informative and entertaining plenary session talk, Malcolm Pike (USC/Norris Comprehensive Cancer Center, Los Angeles, CA, USA) discussed the concept of breast cancer prevention through hormonal manipulation (eg early full-term pregnancy or use of the oral contraceptive pill). This theme was followed up in a subsequent minisymposium in which a number of animal studies that examined the timing of oestrogen exposure in breast cancer risk were presented. Ana Cabanes (Georgetown University, Washington, DC, USA) showed that prepubertal exposure of rats to oestradiol significantly reduced the incidence of mammary tumours in 9,10-dimethyl-1,2-benzanthracene-treated rats. This could be related to expression of specific oestrogen receptor (ER) subtypes: in these rats, ER α appears to be lost temporarily with

increased expression of ER β . In three related studies from the laboratory of Satyabrata Nandi (University of California, Berkeley, CA, USA), short-term hormone treatment appeared to be effective in mammary cancer prevention in rodents, which may be due to alterations in mammary epithelial cell signalling pathways resulting in a reduced proliferative response during carcinogenesis.

Moving on to steroid receptors, Suzanne Fuqua (Baylor College of Medicine, Houston, TX, USA) provided an overview of ERs as targets in breast cancer. This theme was expanded by Rachel Schiff of the same institute and recipient of an AACR Susan G Komen Breast Cancer Foundation Young Investigator Scholar Award, who described expression of wild-type and variant forms of ER β in breast tumours using monoclonal and polyclonal antibodies developed in-house. This is an important development because most previous work on ER β has been at the mRNA level. Analysis of breast tumours revealed nuclear ER β immunoreactivity, but there were no associations with known clinical prognostic markers, highlighting the fact that ER β may have a distinct biological role and is not just a surrogate for ER α . Cell line work showed a differential response to 17 β -oestradiol depending on which ER subtype was expressed, further emphasizing the distinct biological roles of the α and β receptors. Exon 5-deleted ER β variants were also detected in cell lines. This was expanded further by Eli Gilad (Lawrence Berkeley National Laboratory, Berkeley, CA, USA) who showed that expression of this variant acts as a dominant-negative mutant to ER β .

In an eloquent talk by Heather Cunliffe (National Human Genome Research Institute, Bethesda, MD, USA, and recipient of an AACR-Ortho Biotech, Inc, Young Investigator Scholar Award), the role of the steroid receptor coactivator AIB1 was discussed. AIB1 is a receptor coactivator of the p160 family, which is amplified and overexpressed in 10% of breast cancers. Using a range of ER α ⁺ cell lines, recruitment of AIB1 into ER α -oestrogen response element complexes was demonstrated, emphasizing the functional significance of this coactivator in breast tumours with AIB1 amplification.

cDNA array technologies

The rapid evolution of cDNA microarray technology was evident in a number of oral and poster communications covering a range of different gene families. There was tremendous interest in this technology in an educational session describing the design and analysis of microarray experiments, as well as its clinical application.

Outi Monni (National Institute of Health, Bethesda, MD, USA) has applied this approach to characterize the molecular consequences of overexpression of 17q23. Chromosome 17 is one of the most frequently amplified genes in

breast cancer, and harbours several cancer-associated genes, including the tumour suppressor gene *p53*, *BRCA1* and *c-erbB2*. An expression survey of gene transcripts revealed amplifications of *RAD51C*, *S6K*, *PAT1* and *TBX2*, as well as a novel highly amplified expressed sequence tag on the 17q23 amplicon. Together with better-characterized genes, these candidates may contribute to breast cancer development. There were also examples of coupling this technique with another emerging technology, laser capture microdissection, which illustrates its sensitivity.

In the late-breaking abstract section, John Bartlett (University of Glasgow, Glasgow, UK) described the use of genomic microarrays to detect copy changes in microdissected breast tumours. In contrast to standard comparative genome hybridization (CGH) protocols, this 'CGH-on-a-chip' has the added benefit of defining specific regions that are reported to be amplified in breast tumours, and has widespread application in determining the molecular events that underlie disease progression and patient outcome.

Clearly, this is a burgeoning area; identification of novel genes associated with the genesis of breast cancer will surely lead to a better understanding of the processes involved, which may have potential for developing novel treatment strategies in the future.

Microenvironmental contributions to tumour progression

A growing number of investigators are recognizing that, in addition to the chromosomal abnormalities and genetic lesions that destabilize homeostatic cellular function, tumour progression is largely influenced by the tissue microenvironment, that is, the matrix and cellular components of a given tissue type.

Mina Bissell (Lawrence Berkeley National Laboratory, Berkeley, CA, USA) described the mammary epithelial cell models used in her laboratory to understand the mechanisms by which cells normally perceive the surrounding microenvironment and how, as cells become tumorigenic, they lose their ability to sense and respond appropriately to cues in the surrounding milieu.

Recent work showed that effective communication between a cell and its microenvironment is regulated not only by the coordinate activity of cell-surface receptors, such as β_1 integrin and epidermal growth factor receptor, but also by changes in nuclear architecture, as was demonstrated in experiments probing the functional contributions of the nuclear matrix protein NuMA. The importance of microenvironment in tumour cell metastasis was addressed by Anne Chambers (London Regional Cancer Center, London, Ontario, Canada), who discussed a

unique video microscopy system employed to monitor the efficiency of tumour metastasis *in vivo*. Her work shows that metastasis is a highly inefficient process that is dependent on the ability of cells to localize to an appropriate growth-promoting environment. Another dramatic example of the microenvironmental influences on tumourigenic behaviour was described in an early morning 'meet-the-expert' session by Gerald Cunha (University of California, San Francisco, CA, USA), who described experiments exploring stromal-epithelial cell interactions in the context of a prostate tumour progression model. Kidney capsule implants containing mixtures of normal epithelial cells and carcinoma-derived fibroblasts (but not normal fibroblasts) gave rise to dramatic tumour growth, demonstrating that alterations in the stromal constituents of an epithelial tissue microenvironment can have a profound effect on tumour progression.

Considerable interest was generated from a presentation by Miaw-Sheue Tsai (Lawrence Berkeley National Laboratory, Berkeley, CA, USA) who provided potential new insights into the mechanisms by which breast tumours acquire endocrine resistance. By introducing Cyr61, a ligand for $\alpha_v\beta_3$ integrin, into ER⁺ MCF-7 cells, she demonstrated loss of oestrogen-dependence, a growth advantage under serum-free conditions and an invasive outgrowth pattern in Matrigel. Cyr61 may thus be a key factor in controlling tumour growth and progression, perhaps via the integrin pathway.

Angiogenesis

Disruption of tumour vasculature is now recognized as a potentially powerful anticancer strategy. Erkki Ruoslahti (The Burnham Institute, La Jolla, CA, USA) discussed how, using a phage display approach, they identified a series of peptides that home specifically to the microvasculature. By coupling these peptides to known toxins they have developed a strategy for targeting and destroying newly forming blood vessels in developing tumours. They have also characterized a prostate-specific homing phage that targets receptors in normal tissue; normal mice treated with this prostate-specific phage experience extensive cellular apoptosis in the gland and an overall reduction in prostate size. Thus, this strategy might be a viable alternative to surgical intervention in patients diagnosed with hypertrophic prostate glands.

The strong antiangiogenic properties of endostatin make this an attractive candidate for antiangiogenic therapy, but its short half-life makes its effective delivery difficult. To overcome this, an encapsulated polymer system was described by Tatsuhiro Joki (Harvard Medical School, Boston, MA, USA) as a potentially new way of delivering bioactive endostatin. BHK cells were transfected with an endostatin expression vector, and clones expressing high levels of the protein were selected. These were encapsu-

lated in an alginate-polylysine matrix and biologically active endostatin (ie that retained its ability to inhibit angiogenesis) was produced for up to 4 weeks. The use of encapsulated local delivery systems could provide a promising therapeutic approach not only for endostatin, but for other anticancer agents as well.

Groups from the UK and Japan discussed two new angiogenesis inhibitors with clinical potential. Preliminary *in vitro* data with the tubulin-binding agent ZD6126 (ANG 453) showed selective damage to tumour vasculature and widespread necrosis in a range of tumour xenografts, whereas ER-68203-00 also had potent antiangiogenic effects. The broad-spectrum angiogenesis inhibitor, SU6668, which is currently in phase 1 clinical trials, was shown by Douglas Laird (Sugen, San Francisco, CA, USA) to have growth inhibitory effects against a range of newly implanted and established xenografts of different tumour types. As SU6668 inhibits signalling from the vascular endothelial growth factor, fibroblast growth factor and platelet-derived growth factor receptors, its inhibitory effects probably impact both on paracrine and on autocrine pathways, likely involving multiple cell types. In a provocative and controversial talk, Mary Hendrix (University of Iowa College of Medicine, Iowa City, IA, USA) described studies that demonstrated that tumour cells derived from uveal melanomas have the capacity to form blood-bearing channels that are distinct in morphology and behaviour from canonical endothelial-lined angiogenic vessels. In addition to elucidating a potentially novel mechanism of tumour-dependent angiogenesis, these studies provide an excellent example of how even tumour cells, in response to cues from the tissue microenvironment, can adopt an altered phenotype *in vivo*, perhaps masquerading as endothelial cells.

Other molecular targeting strategies

A number of additional targeting strategies were presented at this meeting, some of which may not only increase the efficacy of drug delivery, but may also facilitate drug intervention at localized sites in the body.

Ellen VITETTA (UT Southwestern Medical Center, Dallas, TX, USA) discussed the status of targeted antibody therapies, including the potential for increased efficacy of cross-linked antibodies. For example, recent studies in her laboratory have shown that treatment of lymphoma cells with covalently cross-linked P-glycoprotein antibodies significantly enhances toxic effects of the chemotherapeutic agent doxorubicin. Dr Vitetta also described improved strategies for mouse/human chimeric antibody design by genetic engineering and predicted that future advances will probably involve the development of antibody mimetic molecules that have increased therapeutic efficacy in comparison to the traditional antibody approaches of today.

Bioavailability was also the concern of Stephen Dowdy (Washington University, St Louis, MO, USA) who described a molecular delivery system based on an 11-amino-acid sequence, derived from the HIV Tat protein, which is sufficient to mediate protein transduction in a receptor-independent manner across cell membranes. When injected into mice, purified Tat-tagged-recombinant proteins (ie Tat–LacZ fusions) can be detected in solid tissues, tumours and in blood cells in less than an hour. Based on the success of these proof-of-principle studies, the Tat-dependent transduction system is now being developed for the delivery of tumour suppressor proteins, such as p53 and PTEN, and chemotherapeutic agents, such as doxorubicin, to tumour tissues.

The use of Tat-coupled DNA molecules may even be a viable approach for gene therapy. Roland Burli (California Institute of Technology, Pasadena, CA, USA) outlined the design and use of membrane-permeant gene-specific DNA-binding ligands to attenuate expression of cancer-promoting oncogenes such as HER2/neu.

Finally, work from the group of Brian Druker (Oregon Health Sciences University, Portland, OR, USA) described the development of a small molecule inhibitor, STI571, in treating patients diagnosed with chronic myeloid leukaemia, a disease in which 95% of patients express a constitutively activated Bcr-Abl tyrosine kinase. STI571 shows potent inhibitory activity against the Bcr-Abl kinase *in vitro*, and phase 1 clinical trials show complete haematological responses to STI571 treatment with no detectable toxicity in patients heavily pretreated with conventional therapies. STI571 will now be tested in treatment naïve patients in combination with the standard no-tumour selective approaches. The dramatic efficacy observed in toxicity (phase 1) studies portend that this small molecule kinase inhibitor will become part of standard care in this disease.

Epigenetics and cancer

Clearly, genetic mutation is a fundamental mechanism by which gene expression and function are altered, thereby giving rise to aberrant or cancerous behaviour in cells and tissues. However, many are now discovering that mutation-independent, epigenetic mechanisms, including DNA methylation and histone acetylation, are also critical determinants of tumour progression when they are aberrantly regulated.

Adrian Bird (University of Edinburgh, Edinburgh, UK) gave an overview talk on the role of DNA methylation and histone acetylation status in gene silencing. It now appears that a variety of tumour suppressor genes, including those that encode E-cadherin, *VHL* and *hMLH1*, are inactivated in cancer not by canonical gene mutation, but rather by methylation-dependent promoter silencing.

Because promoter hypermethylation is potentially reversible, the molecules that regulate methylation status of DNA are considered promising targets for new cancer therapies. As a proof of this principle, Paula Vertino (Emory University, Atlanta, GA, USA) described the identification and characterization of *TMS-1*, a gene that is hypermethylated in a variety of breast carcinoma cell lines and primary tumours. Treatment of the cell lines with the methyl transferase inhibitor 5azaC reverses the methylation pattern of *TMS-1* and restores expression of the *TMS-1* molecule.

Joseph Costello (University of California at San Francisco Cancer Center, San Francisco, CA, USA) has used an alternative strategy in developing a broad-based screen to identify candidate CpG islands and to examine differential methylation at these sites between nonmalignant and tumour tissues. Not only is this approach useful for the identification of new epigenetically regulated genes, but this type of analysis may also be applicable for diagnostic purposes, because the observed patterns may be indicative of particular tumour types.

The protein components that control methylation and histone deacetylation were explored in depth in another symposium entitled 'The Cancer–Chromatin Connection'. Using the transcriptionally repressed gene, *MDR1*, as a model, Alan Wolffe (National Institute of Health, Bethesda, MD, USA) presented studies that illustrate the intimate relationship between histone acetylation and DNA methylation in the control of gene expression. Although treatment of cells with either the demethylation inhibitor 5-azacytidine, or the histone deacetylase inhibitor trichostatin A was not sufficient to reactivate *MDR1* expression, a cocktail of both inhibitors acts synergistically to dramatically induce *MDR1* transcription. Reinduction of *MDR1* gene expression is accompanied by a reduction in the methyl CpG binding protein MeCP2, and by the reduced acetylation of histones.

The complexity and significance of chromatin remodelling in controlling cellular behaviour was further illustrated by Douglas Dean (Washington University School of Medicine, St Louis, MO, USA), who showed that unphosphorylated Rb forms transcriptional repression complexes with histone deacetylase and the nucleosome remodelling unit SWI/SNF, or alternatively with just SWI/SNF. Each of these complexes control distinct checkpoints at G1- and S-phase by regulating the ordered expression of the cyclins that ultimately drive the progression of the cell cycle.

Conclusion

Overall, this was an excellent meeting, covering a variety of aspects of clinical and laboratory breast cancer research. We have provided an overview of what we believe to be the most significant contributions. For those

who wish to obtain further details, abstracts are available on-line via the AACR web page (www.aacr.org/). The next annual AACR meeting will be held during March 24–28, 2001, New Orleans, LA, USA.

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