Available online http://breast-cancer-research.com/content/3/2/118

Meeting report

Hormones and Cancer 2000

Elizabeth Anderson

Christie Hospital NHS Trust, Manchester, UK

Correspondence: Elizabeth Anderson, Head of Tumour Biochemistry Laboratory, Clinical Research Department, Christie Hospital NHS Trust, Wilmslow Road, Manchester M20 4BX, UK. Tel: +44 161 446 3221; fax: +44 161 446 3211; e-mail: eanderson@picr.man.ac.uk

Received: 27 November 2000
Accepted: 30 November 2000
Published: 22 December 2000

Breast Cancer Res 2001, **3**:118–121

(Print ISSN 1465-5411; Online ISSN 1465-542X)

Introduction

The Hormones and Cancer 2000 Meeting was held in the spectacular setting of Port Douglas on the North Queensland Coast, Australia, 3–8 November. The meeting comprised three plenary lectures and 11 sessions, two of which were conjoint with the International Aromatase Meeting being held at the same time. In addition to the oral presentations, 50 posters were presented over two lunchtime sessions. There were about 80 registrants for a meeting that was possibly one of the most laid back that I have ever attended, and where even the speakers wore the uniform of shorts, T-shirts, thongs and umbrella required by the rainy season of tropical North Queensland. Despite the relaxed atmosphere, or possibly because of it, the presentations and following discussions were of an extremely high standard. This was also the only meeting that I have ever attended that had a session devoted to a horse race! Yes, we joined the rest of Australia in watching the Melbourne Cup, which, for those who are interested, was won by Brew with novice jockey Kerrin McEvoy on board.

Returning to the science, the meeting focused on recent advances in breast and prostate cancer, with particular emphasis on the role of steroids and steroid receptors in the progression, treatment and prevention of tumours. The speakers and their audience consequently encompassed all disciplines, both scientific and clinical.

Steroid receptor co-regulators

Steroid receptor co-regulators were a major theme of the meeting. There was a session devoted wholly to these proteins and they put in several appearances at other symposia, reflecting their emerging importance in hormonal carcinogenesis, tumour progression, hormone resistance and as therapeutic targets. The first plenary lecture was given by Kathryn Horwitz (University of Colorado School of Medicine, Denver, CO, USA), who presented data on the role of steroid receptor co-activators and/or co-repressors in mediating resistance to antagonists such as tamoxifen or the anti-progestin RU486. Using an elegant strategy in which the ligand binding domain of the progesterone receptor (PR) linked to RU486 was used as bait in a yeast two-hybrid assay, Professor Horwitz' group isolated the co-activator L7/SPA. This co-regulator not only interacts with the PR, but more importantly also appears to be involved in mediating the agonist effects of tamoxifen on the oestrogen receptor (ER). Benita Katzenellenbogen (University of Illinois and College of Medicine, Urbana, IL, USA) used a similar strategy in isolating a protein known as repressor of oestrogen action (REA). Like other co-regulators, this REA protein contains the LXXLL motif required for binding to steroid receptors and it inhibits oestradiol–ER complex activity on several different oestrogen responsive promoters. Interestingly, the REA protein can be prevented from interacting with, and thus inhibiting, the ER by sequestration with prothymosin α (PTα) and it may be no coincidence that PTα expression is high in some breast tumours.

In the session devoted completely to receptor interacting proteins, Michael Stallcup (University of Southern California, Los Angeles, CA, USA) and Malcolm Parker (Imperial Cancer Research Fund, London, UK) both talked about the p160 family of co-activators. Michael Stallcup showed that all members of this family interact with the AF2 activating function of steroid receptors via a common LXXLL

cdk = cyclin-dependent kinase; ER = oestrogen receptor; MAPK = mitogen-activated protein kinase; PR = progesterone receptor; PRA = short form of the PR; PRB = long form of the PR; REA = repressor of oestrogen action.

motif so there must be other mechanisms of introducing specificity to the interactions, which may, in time, prove to be targets for therapeutic intervention. Other data presented by Michael Stallcup suggested that p160 family members might be 'primary interacting proteins' whose role is to recruit secondary co-activators such as the histone acetyltransferases or CBP/p300 to the hormone-activated receptor. Malcolm Parker addressed the very important question of how to examine the function of individual co-activators without interference from endogenous co-regulators. Accordingly, he introduced site-specific mutations into various p160 family members such that they could interact with a modified ER incapable of binding to endogenous p160 proteins. This showed that oestrogen-dependent transcription from reporter genes was dependent upon direct recruitment of a p160 protein and that there was functional redundancy among the p160 family. Professor Parker then went on to emphasise that co-regulators such as p160 can mediate ER interactions with other transcriptional pathways. An important example is the *cerbB2* gene promoter, which is suppressed by the ER occupied by oestradiol via the p160 co-activator.

Myles Brown (Dana-Farber Institute and Harvard Medical School, Boston, MA, USA) demonstrated an ingenious approach to determining which endogenous co-regulators interact with steroid receptors and whether they interact in a sequential, combinatorial or parallel manner. This approach, known as chromatin immunoprecipitation, allows analysis of endogenous co-factors that bind to exogenous oestrogen-dependent promoter sequences such as those of the pS2 and cathepsin D genes. This analysis showed that, after oestradiol treatment, the ER and several of the co-activators cycle on and off the promoters in a precise order. For example, the promoter starts to be occupied by ERα 15 min after the start of treatment with occupation being maximal by 45 min, after which the receptor cycles off. The co-activators AIB-1 and PBP show the same pattern of movement on and off the promoter but CBP and pCAF associate later after pol II recruitment and histone acetylation has taken place. Even more interestingly, treatment with tamoxifen induces recruitment of the NCoR and SMRT co-repressors onto the pS2 and cathepsin D promoter sequences in MCF-7 cultured breast cancer cells where the anti-oestrogen is a pure antagonist. These data are in line with those presented by Kathryn Horwitz (University of Colorado School of Medicine, Denver, CO, USA) in her plenary lecture suggesting that tamoxifen sensitivity depends upon tumour cell content of co-repressors such as NCoR and SMRT in relation to co-activators.

Using this recently acquired knowledge of steroid receptor co-regulators, Donald McDonnell (Duke University Medical Center, Durham, NC, USA) and his group developed a novel strategy for finding compounds that disrupt the interactions between ERα, ERβ and their co-activators. This employs a combinatorial phage display approach to identify small peptides that interact directly with the receptors in the presence of oestradiol and that block transcriptional activity. Clearly, the hope is that these peptidomimetics can be developed into a new class of more specific steroid receptor antagonists for the treatment of breast and other hormone-dependent cancers.

Steroid receptor isoforms and structures in relation to function

Another major theme of the conference was the characterisation of receptor isoforms and their structures in relation to function. The most dramatic discovery in recent years has been that of a second oestrogen receptor, the ERβ. It now seems that ERα and ERβ are often co-expressed and that they can interact. In a breathtakingly comprehensive plenary lecture, Jan-Åke Gustafsson (Karolinska Institute, Huddinge, Sweden), expounded his 'yin yang' hypothesis of ERα and ERβ actions formulated after detailed analysis of the phenotypes of mice in which the ERα and ERβ genes had been knocked out (ERKO and βERKO mice, respectively). This hypothesis is that ERβ acts to suppress ERα action, and this is supported by the higher levels of uterine and prostate proliferation seen in the βERKO mice compared with wild type mice. There is considerable interest in the existence of a separate endogenous ligand for the ERβ, and Professor Gustafsson suggested that it might be 5α-androstane-3β,17β-diol (3β-ADIOL). This clearly needs to be investigated further, as do the relative roles of ERα and ERβα in breast tumourigenesis and progression.

The group that generated the ERα or ERKO mice, and subsequently collaborated in creating the βERKO mice, was represented by Ken Korach (NIEHS/NIH, Research Triangle Park, NC, USA). Professor Korach interestingly described a slightly different phenotype for the βERKO mice to that given by Professor Gustafsson in that they were not able to identify any prostatic hyperplasias. In further studies, the ERKO and βERKO mice were crossed to obtain a double knockout or DERKO strain. The phenotype of the DERKO was equivalent, in many respects, to that of the ERKO except for the ovaries, the appearance of which differed significantly from either of the single knockouts implying that both oestrogen receptors are important for the differentiation of this tissue.

Kathryn Horwitz completed her plenary lecture with some new insights into the differential roles of the long (PRB) and short (PRA) forms of the PR. Using cells that had been engineered to express either PRA or PRB combined with microarray technology, Professor Horwitz was able to identify sets of genes regulated by progesterone via each of the PR isoforms. Remarkably, there seemed to be very little overlap between genes regulated by PRA and those whose expression was influenced by PRB. Christine

Clarke (Westmead Institute for Cancer Research, Sydney, Australia) put this into a clinical context by showing that the PRA and PRB isoforms were expressed in relatively equal amounts in the normal breast and uterus. However, this balance was altered in tumours such that they expressed predominantly one form or the other. This disruption seemed, moreover, to be an early event in the normal to malignant progression as it could be detected in premalignant breast lesions such as atypical hyperplasia. Using breast cancer cells engineered to overexpress one or other of the PR isoforms, Dr Clarke also showed that PRA overexpression was associated with changes in the cytoskeleton and cell morphology that might contribute to the malignant phenotype.

Another group interested in receptor variants and mutants in premalignant breast lesions was that of Suzanne Fuqua (Baylor College of Medicine, Houston, TX, USA), who showed a mutation in the ERα gene to be present in 34% of hyperplasias of usual type. The mutation, which involves the border between the hinge and ligand binding domains of the ERα, appears to confer increased sensitivity to oestrogen, possibly by enhancing binding to SRC-1-type co-activators at low levels of hormone.

Some beautiful crystallographic studies of the ERα and ERβ ligand binding domains occupied with a range of agonists and antagonists were presented by Geoff Greene (The Ben May Institute for Cancer Research, Chicago, IL, USA), which emphasise further the importance of structural changes in receptor activation and inhibition. These studies defined the interface between the AF-2 activating function of the receptors and co-regulators, and also showed how the position of helix 12 is altered after binding of different agonists and antagonists. These data go a long way toward explaining why drugs such as tamoxifen have mixed antagonist/agonist effects, and studies of this type should lead to the development of new anti-steroidal compounds of improved specificity.

Consequences of oestrogen receptor activation

Although a lot of time was devoted to how the oestrogen and other steroid receptors are activated after ligand binding, there were several presentations regarding the more downstream consequences of this activation. Benita Katzenellenbogen began by describing experiments in which differential display was used to identify mRNAs upregulated by anti-oestrogens. One such mRNA was that for quinone reductase, the expression of which is enhanced by anti-oestrogens and is repressed by oestradiol. Professor Katzenellenbogen speculated that enhanced levels of enzymes such as quinone reductase might afford protection against carcinogens and, thus, would be suitable targets for novel breast cancer prevention strategies.

One of the effects of treating hormone sensitive breast cancer cells with oestradiol is increased entry from G0 to the G1 phase of the cell cycle and acceleration of progression through G1. Professor Rob Sutherland and his group (Garvan Institute of Medical Research, Sydney, Australia) analysed the effects of oestrogen and anti-oestrogens on the cyclin–cyclin-dependent kinase (cdk) complexes known to be involved in controlling cell cycle progression. Accordingly, treatment of cultured breast cancer cells with specific anti-oestrogens such as ICI 164 384 or ICI 182 780 rapidly decreases *c-myc* and cyclin D1 expression and, subsequently, the activity of the cyclin D1–cdk4 complex. This, in turn, releases the p21CIP1 and p27KIP1 inhibitors of cdk activity so that they are free to associate with and inhibit the activity of cyclin E–cdk2 complexes. This ultimately results in accumulation of hypophosphorylated retinoblastoma protein, sequestration of the E2F transcription factor and inhibition of G1 to S phase progression. Subsequent oestradiol treatment of anti-oestrogen arrested cells reverses these aforementioned changes, allowing G1 progression. Furthermore, the effects of oestrogen treatment can be replicated by increasing cellular content of c-myc or cyclin D1 via an inducible promoter construct. It is only through painstaking dissections of the mechanisms controlling cell cycle progression such as these that we will begin to understand the means by which oestrogens and anti-oestrogens exert their effects, and to understand how some breast cancers become hormone resistant.

Cross talk between signalling pathways

It has become apparent in recent years that there is 'cross talk' between the signalling pathways of the nuclear receptors and those employed by receptors on the cell surface. The significance of these interactions is, as yet, not clear, but it seems possible that they contribute to processes such as anti-oestrogen resistance. Joyce Slingerland (Sunnybrook and Women's College Health Science Centre, Toronto, Ontario, Canada) described interactions between the ER and the mitogen-activated protein kinase (MAPK) pathways in the control of p27KIP1 expression and activity. This inhibitor of cyclin-dependent kinase activity, as already mentioned, is critically involved in anti-oestrogen induced breast cancer cell growth arrest. Accordingly, treatment with oestradiol reduces breast cancer cell content of p27KIP1, which de-represses cyclinE/cdk2 activity and promotes cell cycle progression. It appears, however, that oestradiol exerts its effects on p27KIP1 via both the ER and the MAPK pathways. This conclusion was reached following experiments in which MAPK was shown to be very rapidly activated by oestradiol treatment; indeed, the kinetics of this activation suggest that oestradiol is acting by a nongenomic route yet to be identified. At later time points, however, the effects of oestradiol appear to be mediated by the classical ER pathway. A role for MAPK in modulating anti-oestrogen sensitivity is suggested by experiments

on the LY2 tamoxifen-resistant breast cancer cell line. The MAPK pathway in these cells is constitutively activated, and treatment with a specific MAPK kinase inhibitor restores anti-oestrogen sensitivity. Further proof of principle was provided by an experiment in which a constitutively active MAPK was introduced into hormone sensitive cells. This reduced p27KIP1 content and produced partial anti-oestrogen resistance. Several components of the MAPK pathway are aberrantly expressed or activated in many breast tumours, and this may be an important mechanism of anti-oestrogen resistance.

The inhibitor of cdk activity p27KIP1 is an important example of a protein whose expression and activity is modulated by multiple signalling pathways including steroid receptors. It now appears that steroid receptors themselves are targets of other cell signalling cascades, and Nancy Weigel (Baylor College of Medicine, Houston, TX, USA) discussed the PR in this context. A number of other signalling pathways converge on the PR to alter its phosphorylation status, which can, in turn, enhance agonist-dependent activity and even, in some cases, induce antagonists to act as agonists. For example, activating the protein kinase A pathway by treating T47D breast cancer cells with a cAMP analogue causes the anti-progestin RU486 to act as an agonist. This has been shown to be due to phosphorylation of PRB in particular, and it is thought that this alteration reduces recruitment of the NCoR and SMRT co-repressors to the antagonist-occupied receptor. Using cells engineered to express either PRA or PRB, Dr Weigel showed that components of the MAPK pathway could also preferentially phosphorylate and stimulate the activity of the PR isoforms. The PR has multiple phosphorylation sites and the use of phosphorylation site-specific antibodies allows analysis of the, sometimes, complex phosphorylation patterns. Interestingly, serine 294 is one site that is a target of many of the cell signalling pathways but there are significant differences in the kinetics of phosphorylation by these pathways. For example, activation of MAPK by epidermal growth factor very rapidly phosphorylates Ser 294, whereas steroid-dependent phosphorylation occurs more slowly. Furthermore, although Ser 294 is common to both PR isoforms, it is very poorly phosphorylated in the PRA *in vivo*. Finally, it is not only steroid receptors, but also their co-regulators that can be phosphorylated by protein kinases. MAPK can phosphorylate the co-activator SRC-1, and this can be blocked by an inhibitor of MAPK activation. Mutation of the MAPK-specific phosphorylation sites substantially reduces the efficacy of SRC-1 as a co-activator. It thus appears that any number of cell signalling pathways can modulate nuclear receptor activity either directly or indirectly by altering the activity of co-regulators.

It is becoming clear, having established that signals from cell surface receptors can modulate steroid receptor activity, that nuclear receptors can reciprocate. Bernd Groner (Institute for Biomedical Research, Frankfurt, Germany) described the interactions between the glucocorticoid receptor and stat5, a transcription factor that is the downstream target for cytokine receptors including those for growth hormone and prolactin. Activated GR forms complexes with stat5, which then bind to the stat5 DNA binding site to enhance transcription of cytokine inducible genes. What is interesting is that molecules such as CBP/p300 and NCoR, previously identified on the basis of their binding to nuclear receptors, also interact with stat5; indeed, the amount of available CBP/p300 may be the limiting factor in stat5 activation. Is it possible that steroid receptor co-regulators will turn out to have a much more universal role in the control of gene transcription?

Conclusions

Until recently, our models of steroid hormone signalling through nuclear receptors were comparatively simple affairs that did not really explain how antagonists such as tamoxifen could act as agonists under certain circumstances. The Hormones and Cancer 2000 Meeting demonstrated how far and how quickly our understanding of steroid receptor action has progressed. Every aspect of nuclear receptor signalling turns out to be more complex than imagined, and there have been some real surprises, including the discovery of a second ER, other steroid receptor isoforms with distinct functions, the large number and variety of co-regulatory molecules, and the mutual interactions between steroid receptor signalling pathways and those used by receptors on the cell surface. By elucidating these complexities, we are beginning to arrive at more satisfactory explanations for the dual nature of some steroid antagonists. This report has focused on a few, highly selected experimental areas but the Hormones and Cancer Meeting actually covered topics from the bench to the bedside and back again to give a full picture of how research into basic mechanisms can be translated into new more effective and specific treatments for breast and other steroid-dependent cancers.