Therapeutic Resistance to Anti-Hormonal Drugs in Breast Cancer

Stephen Hiscox · Julia Gee · Robert I. Nicholson
Editors

Therapeutic Resistance to Anti-Hormonal Drugs in Breast Cancer

New Molecular Aspects and their Potential as Targets

Springer

Editors
Dr. Stephen Hiscox
Cardiff University
Welsh School of Pharmacy
Tenovus Centre for Cancer Research
Cardiff University
Cardiff, Wales, UK
hiscoxsel@Cardiff.ac.uk

Dr. Julia Gee
Cardiff University
Welsh School of Pharmacy
Tenovus Centre for Cancer Research
Cardiff University
Cardiff, Wales, UK
gee@cardiff.ac.uk

Dr. Robert I. Nicholson
Cardiff University
Welsh School of Pharmacy
Tenovus Centre for Cancer Research
Cardiff University
Cardiff, Wales, UK
nicholsonri@cf.ac.uk

ISBN: 978-1-4020-8525-3 e-ISBN: 978-1-4020-8526-0

DOI 10.1007/978-1-4020-8526-0

Library of Congress Control Number: 2008938903

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

Foreword

Anti-hormonal drugs are a mainstay in the treatment of breast cancer and have impact in both primary and metastatic disease. A pervading problem, however, is therapeutic resistance which can either prevent initial response to anti-hormonal measures or be acquired during therapy. The molecular mechanisms underlying resistance are increasingly understood and this knowledge is leading to novel therapeutic approaches to more effective treat or delay the appearance of endocrine resistance.

The goal of the recent 3rd Tenovus/AstraZeneca Workshop in Cardiff was to ask "what's new" in endocrine resistance in breast cancer and assess the progress that is being made towards its treatment. This workshop comprised various talks from international experts and round-table discussion, culminating in the 10 articles within this book. The chapters within describe several key aspects of endocrine resistance and include the use of RNA interference screens to identify modifiers of sensitivity to hormonal therapy, the importance of coactivator and corepressor proteins to endocrine response and resistance and elucidating mechanisms of oestrogen receptor re-expression in ER-negative tumours. Furthermore, the intriguing concepts that antihormones themselves may promote adverse cellular features which sustain both an invasive, endocrine-resistant state and modify cellular interactions with the surrounding stroma together with the potential role of cancer stem cells in resistance are presented. Finally, novel therapeutic strategies in breast cancer such as heat shock protein inhibitors and pharmacological targeting of Src kinase are discussed together with a review of current treatment strategies that seek to combine signal transduction inhibitors with endocrine therapies.

The articles here add to our knowledge of molecular events that underlie hormonal resistance and strategies through which resistance may be circumvented. Continued success in this area will without doubt benefit current and future breast cancer patients and reduce the impact this disease has on its millions of suffers worldwide.

Cardiff, Wales — Stephen Hiscox
Cardiff, Wales — Julia Gee
Cardiff, Wales — Robert I. Nicholson

Contents

Contributors

Simak Ali Department of Oncology, Imperial College London, Hammersmith Hospital Campus, London W12 0NN, UK, simak.ali@imperial.ac.uk

Alan Ashworth The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, SW3 6JB, UK, alan.ashworth@icr.ac.uk

Denise Barrow Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3NB, UK, BarrowD@cf.ac.uk

Jill Bayliss Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan Medical Center, Ann Arbor, MI 48109, USA, jbayliss@umich.edu

Robert B. Clarke Breast Biology Group, School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Manchester, M20 4BX, UK, robert.clarke@manchester.ac.uk

Kathleen Diehl Department of Surgery, University of Michigan Medical Center, Ann Arbor, MI 48109-0932, USA, kdiehl@umich.edu

Dorraya El-Ashry Department of Internal Medicine, Division of Hematology/ Oncology, University of Miami Miller School of Medicine, Miami, FL 33136, USA, del-ashry@med.miami.edu

Gillian Farnie Breast Biology Group, School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Manchester, M20 4BX, UK, gillian.farnie@manchester.ac.uk

Heidi M. Fiegl Medical University Innsbruck, Department of Gynaecology and Obstetrics, Innsbruck, Austria, heidi.fiegl@gmail.com

Julia M.W. Gee Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3NB, UK, gee@cardiff.ac.uk

Lindy Goddard Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3NB, UK, GoddardL@cf.ac.uk

Amy Hilger Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan Medical Center, Ann Arbor, MI 48109, USA, ahilger@umich.edu

Stephen Hiscox Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3NB, UK, HiscoxSE1@cf.ac.uk

Sacha J. Howell Breast Biology Group, School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Manchester, M20 4BX, UK, SHowell@picr.man.ac.uk

Iain R. Hutcheson Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3NB, UK, HutchesonIR@cf.ac.uk

Elizabeth Iorns The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, SW3 6JB, UK, Elizabeth.Iorns@icr.ac.uk

Stephen R.D. Johnston Consultant Medical Oncologist, Department of Medicine, Royal Marsden Hospital, Chelsea, London, SW3 6JJ, UK, stephen.johnston@rmh.nhs.uk

Nicola J. Jordan Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3NB, UK, JordanNJ1@cf.ac.uk

Christopher J. Lord The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, SW3 6JB, UK, chris.lord@icr.ac.uk

Richard A. McClelland Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3NB, UK, McclellandR@cf.ac.uk

Liam Morgan Department of Medical Biochemistry & Immunology, Cardiff University, Cardiff, Wales. CF10 3XF, UK, MorganLD1@cf.ac.uk

Robert I. Nicholson Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3NB, UK, Nicholsonri@cf.ac.uk

Ciara S. O'Brien Breast Biology Group, School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Manchester, M20 4BX, UK, Ciara.Obrien@manchester.ac.uk

Marissa V. Powers Signal Transduction and Molecular Pharmacology team, Cancer Research UK Centre for Cancer Therapeutics, Haddow Laboratories, The

Institute of Cancer Research, Sutton, Surrey, SM2 5NG, UK, Marissa.Powers@icr.ac.uk

Victoria E. Shaw Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3NB, UK, ShawV@cf.ac.uk

Chris Smith Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3NB, UK, SmithC@cf.ac.uk

Andrew Stone Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3NB, UK, StoneA@cf.ac.uk

Kathy M. Taylor Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3NB, UK, TaylorKM@cf.ac.uk

Prakash Vishnu Department of Internal Medicine, Detroit Medical Center/Wayne State University, Detroit, MI 48235, USA, pvishnu@umich.edu

Rosemary A. Walker Department of Cancer Studies & Molecular Medicine, RKCSB, University of Leicester, Leicester, LE2 7LX, UK, raw14@le.ac.uk

Martin Widschwendter Department of Gynecological Oncology, UCL Institute for Women's Health, University College, London, UK, M.Widschwendter@ucl.ac.uk

Paul Workman Signal Transduction and Molecular Pharmacology team, Cancer Research UK Centre for Cancer Therapeutics, Haddow Laboratories, The Institute of Cancer Research, Sutton, Surrey, SM2 5NG, UK, paul.workman@icr.ac.uk

Chapter 1
Experimental Endocrine Resistance: Concepts and Strategies

Robert I. Nicholson, Iain R. Hutcheson, Stephen Hiscox, Kathy M. Taylor and Julia M.W. Gee

Contents

Abstract Intensive research has been undertaken in order to understand the mechanisms that underlie the phenomenon of endocrine resistance with a view to identifying biomarkers predictive of antihormonal response and revealing potential therapeutic targets through which resistance may be delayed or prevented. Through these studies it is increasingly apparent that the tumour cells' ability to harness a variety of growth factor signalling pathways to drive proliferation in the presence of endocrine agents plays a major role in promoting a resistant phenotype. Importantly,

R.I. Nicholson (✉)
Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3NB, UK
e-mail: nicholsonri@cf.ac.uk

S. Hiscox et al. (eds.), *Therapeutic Resistance to Anti-Hormonal Drugs in Breast Cancer*, DOI 10.1007/978-1-4020-8526-0_1,

the inappropriate activation of growth factor signalling cascades is now regarded to play a significant role in the promotion of antihormone failure in breast cancer cells and it is becoming clear that anti-hormones themselves can promote the expression of a number of growth factors and their receptors in the drug-responsive phase, which subsequently play key roles in the regulation of tumour growth during the drug-resistant phase. The importance of growth factor signalling in endocrine resistance is further revealed in that a high degree of interaction exists between intracellular signalling pathways downstream of the oestrogen receptor and growth factor receptors, further contributing to the development of an endocrine insensitive state. It is likely that our increasing knowledge in this area will ultimately lead to the development of inhibitory strategies targeted towards suppressing the activity of growth factor signalling pathways and their interplay with the oestrogen receptor to improve the outlook for breast cancer patients.

Keywords Oestrogen Receptor · Growth factor receptors · Cross-talk · Endocrine resistance

1.1 Introduction

It is self evident that the emergence of either de novo or acquired endocrine resistance in breast cancer cells must result from a subversion of the growth inhibitory activity of anti-hormonal drugs. Experimentally, this can take the form of radical cellular changes which drive mitogenic and survival signalling in breast cancer cells independently of oestrogen receptors (ER) and which can therefore operate in either ER positive or negative cells. Alternatively, however, it can also occur via more subtle changes in cellular pathways which facilitate ER signalling in the presence of anti-oestrogenic drugs or in a reduced oestrogen environment, leading to tumour cell growth (Nicholson et al., 2007).

The purpose of this chapter is to define several of the experimental mechanisms that are believed to underpin endocrine resistance in breast cancer cells and thereby provide a framework against which the more recent findings described in subsequent chapters can be viewed. Additionally, an attempt will be made to establish the therapeutic principals which have originated from the experimental studies and which are now being considered clinically as a means of more effectively treating or delaying the appearance of endocrine resistance. The chapter will primarily focus on the molecular cross-talk that exists between ERα and growth factor signalling pathways, an area that is thought to be a major contributor to the development of resistance to anti-hormonal treatments and which is rich in novel therapeutic approaches. For simplicities sake, the more recently identified actions of oestrogens on ERβ are not widely discussed in this chapter since they are considered by most not to be a major stimulus to growth in breast cancer cells. Indeed, their cellular levels fall as breast cancers progress and they may display growth retarding activity through their heterodimerisation with ERα (Hall and McDonnell, 1999).

1.2 Oestrogen Action and its Coupling to Growth Factor Signalling

The capacity of signalling molecules to induce cell growth has its roots in engaging the cell cycle and this is frequently coupled to the promotion of signals which enable cell survival. In many cell types this is achieved through the actions of locally and distally produced growth factors which act through cell surface receptors to drive established growth and survival pathways. In oestrogen dependent tissues, however, oestrogen receptors, alongside growth factors, are also key players in such events where they act as:

(i) Nuclear transcription factors able to directly engage the promoters of oestrogen regulated genes containing oestrogen response elements (EREs);
(ii) Binding proteins able to associate with other nuclear transcription factors to modulate their activity;
(iii) Cell membrane linked proteins able to facilitate growth signalling by enhancing the actions of several signal transduction pathways.

In each instance, the cellular actions of ERα (subsequently referred to as ER) are productively linked to growth factor signalling cascades to orchestrate growth and survival signalling. Interference with such ER signalling using anti-hormonal drugs has both anti-ER and anti-growth factor actions, while aberrant growth factor signalling can sustain breast cancer cell growth in the presence of anti-oestrogens and in an oestrogen withdrawn environment (Nicholson and Gee, 2000).

1.2.1 Interactions of the ER with EREs

The "classical" pathway associated with the cellular actions of oestrogen receptors (ERα) involves them functioning as nuclear transcription factors able to regulate the expression of genes containing oestrogen response elements (ERE) within their promoters. Genes possessing such EREs include a number of growth promoting growth factors (transforming growth factor alpha (TGFα), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), O'Lone et al., 2004), together with several survival factors, such as bcl-2. Importantly, ERs have two major activator functions, AF-1 and AF-2 which often act together to maximise transcriptional events. In some instances, however, substantial ER-regulated gene expression can be achieved through either AF-1 or AF-2 and this can be both promoter and tissue specific (O'Malley, 2005). Such differential responses are of great potential significance to ER action since AF-1 can be activated by oestrogen-independent mechanisms (often termed ligand-independent), while AF-2 responses have a more strict dependence on the presence of ER ligands (ligand-dependent). All aspects of ER signalling, therefore, are not wholly reliant on the presence of oestrogens and a degree of ER activation can be achieved through the activation of AF-1 by

alterative mechanisms. In this respect, it is noteworthy that ER phosphorylation is a critical event in ER activation and several intracellular kinases have been implicated in this process (Bunone et al., 1996; Joel et al., 1998; Kato et al., 1995; Lannigan, 2003). Indeed, ER phosphorylation of key sites within the AF-1 domain is thought necessary for ligand-independent ER transcription and may be achieved, for example, through growth factor induced activation of p42/p44 mitogen-activated protein kinase (MAPK) and AKT, possibly in a c-src dependent manner.

In addition to the ER, transcriptional activity arising from ER activation is modulated through multiple co-regulatory proteins which complex with the ER (Osborne and Schiff, 2005). These proteins can have histone-acetyltransferase (HAT) activity, required for chromatin decondensation (NCoA[nuclear receptor co-activator]1 or SRC1; NCoA2 or TIF2; NCoA3 or AIB1) or can recruit histone-deacetylase complexes (HDAC) to reverse this process (NCoR[nuclear receptor co-repressor]1 & 2) and are termed co-activators and co-repressors respectively. Such opposing actions, for example, can lead to the enhancement of AF-2 activity following the oestrogen-induced recruitment of co-activators or a reduction in AF-2 activity when co-repressors are present. Significantly, phosphorylation of co-activator proteins, alongside the ER, is critical for the activation of ER directed gene transcription and once again can be promoted by growth factor driven protein kinases, including MAPK and AKT (Shou et al., 2004). Clearly, a synergy exists between ER and growth factor signalling, where appropriate cellular growth and gene expression undoubtedly requires the measured activation of each.

1.2.2 Interactions of ER with Other Nuclear Transcription Factors

As stated above, ERs also act as binding proteins able to associate with other nuclear transcription factors to modulate their activity and this is considered to be contributory to oestrogen associated growth responses. They are able to achieve this through direct protein:protein interactions enabling them to effect other DNA regulator sequences and hence the expression of genes which do not necessarily contain EREs in their promoters. Such “non-classical” actions are known to impact on Jun/Fos activator protein 1 (AP-1) and specificity protein-1 (SP-1) sites in DNA and to influence the expression of growth factor receptors (IGF-1R), nuclear transcription factors (myc) and cell cycle regulatory proteins (cyclin D1), key components of proliferation and survival signalling (O’Lone et al., 2004; Shupnik et al., 2004).

1.2.3 Interactions of ER with Other Signalling Elements at the Cell Membrane

The final and most recent mechanism believed to be contributory to the growth promoting actions of ERs involves what has become known as “non-genomic” signalling whereby ERs become associated with the cell membrane through

cytoplasmic membrane anchors (Jacob et al., 2006) and are able to rapidly respond to oestrogens (Losel et al., 2003). Indeed such membrane initiated steroid signalling (MISS) occurs within minutes of oestrogen exposure and does not initially require transcriptional events. The importance of MISS is that it has been reported to interact with and/or activate several growth factor receptors (EGFR (Razandi et al., 2003), HER2 (Chung et al., 2002), IGF-1R (Kahlert et al., 2000)), signalling enzymes (Wong et al., 2002), adaptor proteins (Shc (Song et al., 2002)) and intracellular kinases (MAPK (Zhang et al., 2002), PI3K/AKT (Sun et al., 2001), c-src (Migliaccio et al., 2002)) that are intimately involved in cell growth and survival mechanisms and which are able, as described above, to drive a "feed forward" circuit of ER-induced transcriptional events involving both "classical" and "non-classical" nuclear steroid signalling pathways (Bedard et al., 2008). Indeed, in a recent model of steroid hormone signalling, O'Malley (2005) described membrane ER actions as an important means of activating several protein kinases which aid the actions of co-activators essential to ER mediated transcriptional events. Membrane associated ER effects may, therefore, be viewed as initiating ER signalling within responsive cells and promoting intracellular signalling cascades which augment the later nuclear actions of ERs. As such, membrane and nuclear ER signalling appear complementary in the induction of growth and survival mechanisms.

Interestingly, ER directed "non-genomic" responses (like nuclear ER responses) also rely on co-regulatory proteins that may be influenced by various signal transduction elements and one of these is believed to be over-expressed in some breast cancers. This protein (MNAR [modulator of non-genomic activity of ER]/PELP 1 [proline-, glutamic acid-, and leucine rich protein 1]) enhances ER directed nuclear and membrane signalling (Vadlamudi et al., 2001; Wong et al., 2002) and contains c-src activating domains which directly activate ERK/MAPK when in association with ER (Barletta et al., 2004). Controversially, other membrane associated proteins, such as GPR30, appear also to bind oestrogens with low affinity and instigate some signalling via EGFR transactivation. In breast cancer cells, however, GPR30 knockdown does not effect oestrogen signalling (Pedram et al., 2006) and their contribution of endocrine response and resistance is not considered further.

1.3 Anti-Hormone Action

By definition, all oestrogen targeted endocrine therapies have the common goal of depriving breast cancer cells of their required oestrogenic stimulation and reducing its productive cross-talk with interactive growth factor signalling elements to promote cell cycle arrest and induce cell loss. They achieve this either by lowering circulating oestrogen levels (using LH-RH analogues and aromatase inhibitors), or by antagonising their cellular actions by competition for ERs (using anti-oestrogenic drugs) (Nicholson and Johnston, 2005). Although such distortions of oestrogen mediated signalling have been described in terms of both "genomic" and "non-genomic" responses, characteristically, these actions are not simple and in some

instances vary between procedures associated with oestrogen withdrawal from ERs and those which involve ER occupancy by anti-oestrogenic drugs. Because of this, the different modalities used to treat breast cancer patients will be dealt with separately.

1.3.1 Selective Oestrogen Receptor Modulators (SERMs)

Early pharmacological studies using drugs such as tamoxifen, toremifene and raloxifene quickly established that they possessed mixed agonistic and antagonistic activity and that this varied considerably within oestrogen target tissues such as the breast, uterus and bone. In human breast cancer, SERMs are believed to be predominantly antagonists, although their limited agonistic activity has been linked to the phenomenon of tumour flare (Reddel and Sutherland, 1984) and may be exaggerated by excessive growth factor signalling to form a resistance mechanism (see below). Importantly, while SERMs are generally considered to be effective inhibitors of oestrogen-dependent AF-2 activity, they are considerably less effective on ligand-independent AF-1-mediated transcriptional responses (Tzukerman et al., 1994). The relative expression of AF-1 and AF-2 dependent genes within varying tissues (and potentially within breast cancer samples) may thus go some way to rationalising the mixed agonistic and antagonistic properties of SERMs, as may varying tissue availability of co-activators and co-repressors (Jordan and O'Malley, 2007).

1.3.2 Pure Anti-Oestrogens

The recognition that the agonistic activity of SERMs might limit their anti-tumour efficacy led to the development of a class of anti-oestrogenic drugs which, in many experimental settings, completely lack oestrogen-like properties. These drugs, which are epitomised by the oestradiol analogue fulvestrant (faslodex®), possess a long alkylsulphinyl side chain which disrupts cytoplasmic to nucleus ER translocation, ER dimerisation and binding to DNA and therefore severely limits both AF-1 and AF-2 responses (Osborne et al., 2004). Moreover, they also promote rapid degradation of ER following increased ER ubiquitinisation (Carlson, 2005), a novel property that must limit the capacity of ER to associate with other transcription factors and localise to the cell membrane. Because of their capacity to promote ER loss they are frequently termed selective oestrogen receptor down-regulators (SERDs). As testimony to the improved inhibitory actions of pure anti-oestrogens on ER, they are often considerably more effective than SERMs at promoting anti-tumour effects in several in vitro and in vivo models of human breast cancer (Carlson, 2005) and they often are active in tumours that have acquired a resistance to SERMs and oestrogen deprivation (see below).

1.3.3 Oestrogen Deprivation

Fundamentally, oestrogen deprivation of breast cancer cells differs from their treatment with anti-oestrogens since the latter involves ER occupancy while the former does not. This is not just esoteric since unoccupied ER is largely an inactive molecule bound to heat-shock proteins, whilst on ligand binding such proteins are released and the ER may, in the case of SERMs, be subject to varying degrees of activation. This not only applies to nuclear ERs, but also to membrane localised ER whose activation is believed to be highly dependent on ligands (Levin and Pietras, 2008). Theoretically, therefore, oestrogen deprivation should be a highly effective treatment for oestrogen dependent breast cancer, although it is currently unclear how complete oestrogen loss needs to be to maximise such responses and whether ER occupancy by other ligands (e.g. phyto-oestrogens or androgens) can compensate for the decreased availability of more classical oestrogens.

1.4 Mechanisms Associated with Endocrine Resistance

It is evident from the above that a complex bi-directional cross-talk exists in endocrine responsive breast cancer cells between ER and growth factors to sustain growth and survival signalling and that anti-hormonal drugs are able to differentially effect elements of such signalling to promote growth inhibition (Nicholson and Gee, 2000; Nicholson et al., 2007). Clinically, however, while disease control by anti-hormonal drugs offers disease free and survival benefits, they do not work in all patients and responses in others are at best transitory (Bedard et al., 2008). Experimentally, data is described which indicates that such resistance can occur in ER dependent and ER independent forms through both genetic (De Laurentiis et al., 2005) and drug-induced (Gee et al., 2006) alterations in growth factor signalling within breast cancer cells and more detailed descriptions of its individual components are described below.

1.4.1 ER Dependent Mechanisms: Growth Factor Pathway Switching

1.4.1.1 Anti-Oestrogens

Multiple studies have now shown that enhanced growth factor signalling can support elements of ER activity in the presence of anti-oestrogenic drugs and that such responses can be self perpetuating since the resultant increased nuclear and membrane ER activity can further reinforce growth factor activity through the induction and activation of growth factor signalling elements respectively (Massarweh and Schiff, 2007). Considerable work has been performed in this area using MCF-7 cells genetically engineered to over-express EGFR/HER2, where increased growth factor signalling augments both genomic and non-genomic ER actions in the presence

of tamoxifen, leading to de novo tamoxifen resistance (Benz et al., 1992; Shou et al., 2004). In this model, increased growth factor induced protein kinases, for example, are deemed responsible for the phosphorylation of the AIB1 co-activator which allows some nuclear ER signalling in the presence of tamoxifen (agonism) (Font de Mora and Brown, 2000; Osborne et al., 2003), while high levels of membrane associated EGFR/HER2 facilitate the non-genomic oestrogen-like activities of the tamoxifen/ER complex (Chung et al., 2002; Shou et al., 2004). These data are paralleled clinically, in that the co-expression of HER2 and AIB1 confers a poor outlook to patients receiving adjuvant tamoxifen therapy (Osborne et al., 2003). Significantly, other studies have suggested that additional co-activators, such as NCoA-1/SRC-1, may also promote a resistant phenotype in tumours over-expressing HER2 (Fleming et al., 2004). Clearly, increased ER/growth factor signalling at tumour cell membranes, coupled to the activation of nuclear ER components appears to radically alter the pharmacological properties of SERMs in favour of agonistic and growth promoting activity. Critically, this new and intricate cross-talk is sensitive to fulvestrant (Nicholson et al., 1995; Dowsett et al., 2005) and gefitinib (McClelland et al., 2001, Knowlden et al., 2003) and is much less evident in non-transfected cells which express only modest levels of EGFR/HER2, where the antagonistic properties of tamoxifen predominate.

As mentioned above, a feature of breast cancer cells expressing high levels of growth factor receptors is that they often show evidence of an increased activity of several downstream protein kinases involved in signal transduction. These include MAPK (Knowlden et al., 2003), AKT (Jordan et al., 2004; Beeram et al., 2007), PAK-1 (Holm et al., 2006, Rayala et al., 2006), PKA (Michalides et al., 2004) and c-src (Chu et al., 2007; Hiscox et al., 2006c), which are able to directly or indirectly promote the activation of ER signalling components. They may also show elevated DNA binding activity of transcription factors, such as AP-1 which, depending on the balance of nuclear co-regulators, can use tamoxifen ER complexes to aid signalling through alternative response elements (see Section 1.2.2). Since cells genetically engineered to express highly activated forms of these signalling elements often show de novo resistance to tamoxifen (PAK1, Rayala et al., 2006; AKT, Clark et al., 2002, Beeram et al., 2007;Yoo et al., 2008, MAPK, Donovan et al., 2001), while their inhibition may restore response in acquired resistance models (Knowlden et al., 2003), they clearly play a central role in the development of endocrine resistance and may, in some instances, directly substitute for the over-expression of growth factor receptors. Certainly, elevated activity of MAPK (Gee et al., 2001), AKT (Kirkegaard et al., 2005) and AP-1 (Johnston et al., 1999) in clinical breast cancer samples has been linked to anti-oestrogen resistance.

In addition to a role of EGFR/HER2 in de novo tamoxifen resistance, these growth factor receptors also play a critical part in the development of acquired resistance to tamoxifen in MCF-7 cells (Nicholson et al., 2007). This arises because oestrogens, in addition to inducing several growth factor signalling elements (see Section 1.2.1), also act to repress the expression of others, including EGFR/HER2 (see Gee et al., 2006). Consequently, blockade of ER signalling upregulates EGFR/HER2 expression in a time dependent fashion reaching after 3

months treatment in vitro approximately a 40-fold rise in their membrane expression levels (Knowlden et al., 2003). These dramatic changes are accompanied by an increased formation and activation of EGFR/HER2 heterodimers, which once again serve to drive ER-dependent tamoxifen-resistant proliferation and survival through recruitment and activation of MAPK (Knowlden et al., 2003), AKT (Jordan et al., 2004) and c-src (Hiscox et al., 2006c). In our own studies, phosphorylation of serine 118 within the ER by MAPK appears of key importance, since it allows the recruitment of several co-activators (e.g. p68 RNA helicase) to the tamoxifen ER complex (Britton et al., 2006). Concurrent reporter gene construct studies in tamoxifen resistant cells indicate that EGFR/MAPK-promoted ER/AF-1 phosphorylation enhances the agonistic activity of the tamoxifen/ER complex and re-instigates the expression of several ERE-containing genes. Significantly, this reactivation of ER was found to be associated with the increased production of key ligands that promote EGFR, HER2 and IGF-1R signalling, including transforming growth factor β, amphiregulin, epiregulin and IGF-II, with chromatin immunoprecipitation (ChIP) assays demonstrating that ER is bound to a consensus ERE within the amphiregulin promoter in tamoxifen resistant MCF-7 cells. Critically, neutralising antibody studies against several EGF-like ligands established that ampiregulin is indeed the essential element driving the elevated EGFR/HER2 signalling in these cells (Britton et al., 2006). Although the temporal sequence of these events remains to be established during the development of acquired resistance to tamoxifen, we have postulated that EGFR/HER2/MAPK/ER driven increases in the expression of ampiregulin may serve to establish a self-propagating autocrine signalling loop allowing the emergence and maintenance of efficient EGFR-promoted resistant growth. An additional feature of this loop involves the increased IGF-II production noted above, which facilitates a Src-dependent cross-talk between the IGF-1R and the EGFR (Knowlden et al., 2005). In this model, increased ER driven IGF-II production results in increased IGF-1R promoted Src phosphorylation which then phosphorylates tyrosine 845 on the EGFR to enhance the kinase activity of the EGFR. The activation of this phosphorylation site on the EGFR is necessary for them to respond to EGF-like ligands (Biscardi et al., 1999). Clearly, retained nuclear ER signalling in tamoxifen resistant cells offers a considerable boost to the activation of growth factor signalling elements and is entirely complimentary to an increased redistribution of ER to extranuclear sites in tamoxifen resistance (Fan et al., 2007), where it produces parallel productive interactions with membrane associated EGFR/HER2/IGF-1R leading to the further activation of several signal transduction cascades (Fan et al., 2007; Massarweh and Schiff, 2007). Significantly, c-src appears central to the relocation of ER to the tumour cell membranes as the process is reversed by a src kinase inhibitor (Fan et al., 2007).

Interestingly, although the mechanisms which lead to the induction of EGFR and HER2 in endocrine resistant cells vary at the transcriptional level, they are reported to involve a negative regulatory element within the first intron of their genes (Wilson and Chrysogelos, 2002; Newman et al., 2000). Additionally, however, Hurtado et al., (2008) have implicated the Paired Box gene 2 product (Pax2), in a novel role, as a critical mediator of ER repression of HER2 by oestrogen which paradoxically is

shared by tamoxifen. Critically, however, the capacity of Pax2 to repress HER2 in tamoxifen treated cells is reversed by AIB-1 which competes out Pax2 binding to a HER2 cis-regulatory element, with now AIB-1 driving increased HER2 expression. These data suggest that Pax2 functions as a repressive protein which competes with an activating protein for the regulation of the HER2 gene. As such, either a decrease in Pax2 expression or an increase in AIB-1 levels would overcome the initial repressive effects of tamoxifen on HER2 transcription. Importantly, in our tamoxifen resistant cells although the former appears to predominate, Hurtado et al., (2008) demonstrated that this allows effectively allows more AIB-1 to associate with the HER2 cis-regulatory element to drive increased HER2 expression. Clinically, they also observed that increased Pax2 was associated with lower HER2 expression and with improved survival.

In a recent study, Soni et al. (2008) have demonstrated that tamoxifen resistant MCF-7 cells in vitro also over-express the focal adhesion docking protein encoded by the breast cancer anti-oestrogen resistance-1 (BCAR-1, also known as p130cas) gene. BCAR-1, first identified by Dorssers et al. (1993) using a functional assay to detect genes involved in oestrogen-independent growth of breast cancer cells, has several important cellular functions, including an ability to aid membrane ER signalling (Cabodi et al., 2004), together with a capacity to relocate the guanine nucleotide exchange factor (GEF) BCAR-3/AND-34 to the cell membrane where it activates numerous small GTPases (Cai et al., 2003). Critically, Soni et al. (2008) demonstrated that blocking the activity of BCAR-1 in tamoxifen resistant cells reduced EGFR levels and attenuated EGFR signalling onto ERK and PI3K/AKT, leading to an inhibition of cell proliferation and increased apoptosis i.e. re-sensitises the cells to the growth inhibitory actions of tamoxifen. Evidently, BCAR-1 is an essential element in regulating growth factor driven signalling in this model of tamoxifen resistance, an observation concordant with the report of its increased expression in human breast cancers where patients have a reduced overall survival and intrinsic resistance to tamoxifen (van der Flier et al., 2000). BCAR-1 also docks with c-src kinase leading to the phosphorylation and activation of both src and BCAR-1 (Soni et al., 2008). BCAR1/C-src kinase complexes, therefore, appears to play a dual role in promoting EGFR signalling, firstly by directly phosphorylating tyrosine 845 in the EGFR and secondly by enabling growth factor downstream signalling through the activation of several small GTPases.

Significantly, over-expression of BCAR3 in ZR-75-1 breast cancer cells also readily confers anti-oestrogen resistance and detailed evaluation of its downstream signalling components has shown that it activates several Rho family GTPases, including Cdc42 and Rac leading to increased kinase activity of the Cdk42/Rac-responsive serine/threonine kinase PAK-1 and cyclin D1 promoter activation (Cai et al., 2003). In this study, Cai and his colleagues also showed an increased association of BCAR3 and BCAR1 in 578-T cells, an oestrogen independent cell line, and demonstrated that loss of anti-oestrogen response in ZR-75-1 cells was recapitulated by transfection of a constitutively active form of Rac1, supporting a critical role for BCAR1, BCAR3 and Rac1 in anti-hormone resistance.

Finally, over-expression of several growth factors has been show to promote tamoxifen resistance (and/or oestrogen independence) in breast cancer cells in vitro and in vivo. These notably include PC cell-derived growth factor (Tangkeangsirisin et al., 2004), also known as progranulin, vascular endothelial growth factor (Guo et al., 2003), which stimulates breast cancer cell proliferation in vitro, together with angiogenic responses in vivo, keratinocyte growth factor (Chang et al., 2006), whose capacity to override the inhibitor actions of tamoxifen in endocrine responsive MCF-7 cells is reversed by silencing of the keratinocyte growth factor receptor (Rotolo et al., 2008) and heregulins (Tang et al., 1996), which promote the formation of HER3/HER2 heterodimers and strongly promote growth and survival pathways through the activation of MAPK and Akt (Hutcheson et al., 2007). Interestingly, Folgiero et al. (2008) have recently shown that $\alpha 6\beta 4$ integrin is also capable of inducing HER3 in breast cancer cells to maintain the PI3K/Akt survival pathway and tamoxifen resistance, while Liu et al. (2007) have shown that HER3 silencing abrogates HER2-mediated tamoxifen resistance via the inactivation of the PI3K/Akt pathway. Taken together with the parallel identification of colony-stimulating growth factor, fibroblast growth factor 17, platelet derived growth factor receptor α and β, Akt1 and Akt2 as signalling molecules able to promote resistance following retroviral insertion mutagenesis (Meijer et al., 2006; van Agthoven et al., 2008), clearly indicate that there are many potential ways to achieve breast cancer growth in the presence of anti-hormonal drugs. The likely common denominator, however, being, the recruitment and activation of signalling transduction cascades which drive growth and survival signalling.

1.4.1.2 Oestrogen Deprivation

An important distinction between the cellular actions of anti-oestrogenic drugs and oestrogen deprivation is that the latter invariably promotes substantial increases in ER levels which appear particularly sensitive to altered growth factor signalling. This has been described in several breast cancer models employing oestrogen deprivation and can lead to adaptive hypersensitivity to oestrogens and resistant growth (see review by Nicholson et al., 2004). Once again, both membrane-initiated steroid signalling (MISS) and nuclear initiated steroid signalling (NISS) have been implicated in this form of resistance, with increased signalling through MAPK and AKT being provided by increased levels of HER2 and IGF-1R.

Significantly, the oestrogen deprived model used by Richard Santen's group is highly dependent on the increased levels of membrane associated ER being activated by minute levels of oestrogens (10^{-13}M), leading to the rapid growth factor dependent activation of the Ras/Raf/Mek/MAPK and PI3K/AKT signalling cascades which then promote increased nuclear signalling events at the level of cell cycle regulators, such as E2F1 (Yue et al., 2007). Provocatively, however, in another model of oestrogen hypersensitivity, greater emphasis is placed on the capacity of ER and growth factor directed pathways to converge on the regulation of nuclear ER activity which is dependent upon MAPK, p90RSK and AKT (Martin et al., 2005). Despite

these differences, cross-talk between ER and growth factor signalling elements once again underpin the resistant states since the cells remain sensitive to fulvestrant and appropriate signal transduction inhibitors. Interestingly, studies from our own group have produced a third model of resistance to oestrogen deprivation which does not gain adaptive hypersensitivity (Staka et al., 2005). Although this model, unlike those described above, is derived from breast cancer cells cultured in a reduced oestrogen and growth factor environment, shows no evidence of using EGFR/HER2 and IGF-1R signalling, ER and AKT remain critical to the growth of the cells and productive cross-talk between these elements is suggested by inhibitor studies.

1.4.1.3 Anti-Hormone Induced Changes in Growth Factor Signalling

Recently Gee et al. (2006) has evaluated in some detail the capacity of anti-oestrogens to induce gene expression during the early phase of their inhibitory response and has concluded that multiple genes, alongside EGFR/HER2, may attenuate growth inhibition leading to anti-hormone resistance, including NFkB, Bag1, 14-3-3, and tyrosine kinases, such as Lyn (Gee et al., 2006; see also Chapter 4). Interestingly, additional induced genes appear to confer other adverse features to the breast cancer cells in an appropriate cellular environment, with CD59 facilitating evasion of immune surveillance and RhoE, αcatenin and c-src promoting a more invasive phenotype when intercellular contacts are compromised. These data may go some way to explain the emerging relationship between the development of resistance and the gain of a more aggressive breast cancer phenotype (Hiscox et al., 2006c; also see Chapter 8).

1.4.2 ER Independent Endocrine Resistance

Based on the above, exaggerated ER/growth factor cross-talk can play a very dominant role in the development of several experimental forms of endocrine resistance. However, there is also experimentally derived data demonstrating that when more extreme, aberrant growth factor signalling can drive tumour cell growth in a manner dislocated from steroid hormone receptors. As such several mechanisms may contribute to ER independent signalling, including genetic or phenotypic changes that alter the expression of key genes effecting growth factor signalling. Certainly, the original study by Dorrsers and colleagues (Dorssers et al., 1993, van Agthoven et al., 1998) identified BCAR-1 and BCAR-3 as genes that were able to support resistant growth in an ER-independent manner, presumably by maximising the efficiency of growth factor signalling. This concept is supported by a more recent study by Riggins et al. (2006) who described the capacity of BCAR-1 to dock with c-src, an interaction which led to the phosphorylation and activation of both proteins and an activation of the EGFR (via phosphorylation of tyrosine 845 on the EGFR) in manner that did not require ER signalling. Indeed, in that study they also described a