Commentary

Bringing estrogen receptors under control

Kathryn B Horwitz

University of Colorado Health Sciences Center, Denver, Colorado, USA

Received: 5 August 1999
Accepted: 5 August 1999
Published: 27 August 1999

Important note about how to cite this article
This article is also available online in the *Breast Cancer Research* website. To avoid confusion, please ensure that only the online version of the article is cited in any reference, as follows:
Horwitz KB: Bringing estrogen receptors under control [commentary].
http://breast-cancer-research.com/vol1no1/27aug99/dispatch/2

A new term, 'selective estrogen receptor modulator' (SERM), has infiltrated the estrogen receptor (ER) literature lately [1]. It is nothing more than the reaffirmation of an old fact, namely that different estrogens have different effects, in different tissues. The major natural estrogens – estradiol, estriol and estrone – bind ERs with differing affinities, hence variations in their tissue distribution and concentrations influence the extent of their estrogenic effects. Studies with synthetic estrogens have focused on antiestrogenic ligands, which bind ERs and interfere with the actions of the natural estrogens. Tamoxifen is the prototypical antiestrogen, and newer second-generation antagonists, such as raloxifene, are in various stages of clinical trials [2]. Both tamoxifen and raloxifene are SERMs, because their antiestrogenic effects are restricted to only certain tissues. Tamoxifen has been used for more than 20 years to treat ER-positive breast cancers [3]. It was first demonstrated to be effective in advanced disease, later in adjuvant settings, and most recently as a breast cancer preventant in women at high risk. Thus, in various settings tamoxifen is an inhibitory ER ligand in the breast, and this property explains both its efficacy and its widespread use.

The picture is not all rosy, however. True to its SERM nature, tamoxifen is not antiestrogenic in all tissues. For example, in the uterus tamoxifen is a potent estrogen, where, like estradiol (when unopposed by progestins), it induces epithelial hyperplasia and endometrial cancers [4]. The excitement surrounding raloxifene stems from the fact that, like tamoxifen, it is an antagonist in the breast, but, unlike tamoxifen, it lacks estrogenic activity in the uterus [2,5]. In summary, tamoxifen can be either an agonist or an antagonist in normal tissues.

Unfortunately, the same duality of function operates in malignant tissues, including breast cancers. Almost without exception, breast cancers that initially respond well to tamoxifen by growth cessation or regression eventually resume growing despite the continued presence of the antagonist. How can this 'acquired resistance' be explained? Most tamoxifen-resistant tumors continue to express ER [6], suggesting that resistance is not simply due to outgrowth of a nonresponsive, ER-negative subpopulation. Indeed, tamoxifen-resistant tumors remain responsive to growth inhibition by pure antiestrogens (but clinical data are sparse) and other hormonal therapies [3,5]. Paradoxic reports of tumor stasis and even regression after tamoxifen withdrawal in resistant patients [7] suggest that in at least some resistant tumors the antagonist has switched to an agonist. Thus, for several years the notion has been advanced that the term 'resistance' inappropriately describes such tumors, and that tamoxifen is not simply inactive (as implied by the term 'resistance), but, instead, that it has switched to an agonist, and actively stimulates tumor growth [8,9].

That the same ligand can have opposing transcriptional and biologic effects has long been puzzling, but recent advances in our understanding of the molecular biology of steroid receptors has shed light on this paradox. We now know that transcriptional regulation by liganded, DNA-bound receptors is influenced by their association with multiprotein activator or repressor complexes. Detailed analyses of the identity and function of the constituent 'coregulatory' proteins in these complexes are being carried out in many laboratories. They break down into two classes – coactivators and corepressors – and involve proteins with a variety of functions, including the follow-

ER = estrogen receptor; SERM = selective estrogen receptor modulator.

ing: enzymes such as acetylases, deacetylases, methyltransferases, ubiquitin ligases, proteases, ATPases and kinases; proteins with activator or repressor domains that stabilize or destabilize protein–protein interactions; scaffolding proteins involved in the assembly of multiprotein complexes; and even nonpeptide factors such as the steroid receptor RNA activator [10,11].

What does this have to do with tamoxifen? It turns out that the activity of the tamoxifen–ER complex can be exquisitely modulated by the nature of the associated coregulatory proteins. Binding of corepressors, such as the silencing mediator for retinoid and thyroid receptors or nuclear receptor corepressor, suppresses the partial agonist activity of tamoxifen. At least one antagonist-specific coactivator, the L7 switch protein for antagonist, enhances the partial agonist activity of tamoxifen [9]. As a result of these basic molecular studies, there is now intense interest in correlating tamoxifen resistance in breast cancer with the underexpression of corepressors or the overexpression of coactivators. These proteins could clearly represent the next targets for therapeutic interventions. Additionally, although we have learned a great deal about steroid receptor coregulatory proteins in recent years, most investigators believe that only a minor subset have been identified to date. This is because the many subtle structural variations in the conformation of receptors that result from the binding of different ligands yield multiple subtly different targets on the receptor's surface for the binding of a variety of coregulators. It is this variability that can, in part, explain the tissue specificity and paradoxic agonist activity of ligands like tamoxifen.

The hunt is therefore also on to identify the large number of endogenous coregulatory proteins that are probably lurking in tissues, and, additionally, to synthesize their pharmacologic equivalents with a view to manipulating the functional direction of ligand–receptor complexes. In a recent paper, Norris *et al* [12] described a novel method to define an array of synthetic peptides that interact specifically with estradiol- or tamoxifen-occupied ER, and regulate their transcriptional activity. Several methods have recently been developed to select members of random peptide libraries based on their binding affinity to known protein targets [13]. In the method of phage-display, a library of phage, each displaying a different cloned peptide sequence on its surface, is exposed to a plastic plate coated with the target protein. Specifically bound phage are eluted, the phage are amplified, and the process is repeated for several rounds, after which the selected clones of interest are isolated from the phage, the DNAs are sequenced, and the peptides they encode are deduced. Norris *et al* [12] used tamoxifen- or estradiol-occupied ER as the target protein bound to the plate, and they ensured that the receptors would be in the appropriate DNA-bound structural conformation by precoating the plastic with DNA containing estrogen response elements. The screen led to the isolation of several, 15 amino acid peptides, representing three major classes: α/β I, which interacts with estradiol-occupied ER; α/β III or V, which interact with tamoxifen-occupied ER; and α II, which interacts with ER in the presence of either ligand, in the presence of a pure antiestrogen, and even in the absence of ligand.

The α/β I peptide SSNHQSSRLIELLSR interacts with ER only in the presence of estradiol, and not in the presence of SERMs like tamoxifen, raloxifene, GW7604, idoxifene, nafoxidene or the pure antiestrogen ICI182,780. In the presence of agonists, it also interacts with the progesterone receptor B-isoform, and glucocorticoid receptors. When overexpressed, α/β I and α II peptides reduce the transcriptional activity of estradiol, whereas α/β III or V have no effect, which is consistent with their inability to bind ER in the presence of the agonist. On the other hand, peptides α/β III or V are quite tamoxifen-specific for ER, but also bind antagonist-occupied progesterone receptors. Six peptides of the α/β V class were isolated, that had the consensus sequence (S/M)X(D/E)(W/F)(W/F)XXXL. α/β III or V, as well as α II, inhibit the partial agonist effect of tamoxifen, but do not alter transcription by estradiol-occupied ER. The inhibitory activity of these synthetic peptides thus resembles that of the natural corepressors SMRT and N-CoR [9]. It would be of interest to determine whether the complementary DNAs encoding these synthetic peptides could be used as probes to isolate additional endogenous corepressors from complementary DNA libraries. At present the list of known corepressors is much smaller than that of known coactivators [11], and it is unclear whether this discrepancy represents a true cellular condition, or whether it is an artifact due to the technical complexity of screening for corepressors.

Norris *et al* [12] speculated that each class of peptides recognizes different protein contact sites on the ER protein; contact sites that are generated specifically by the class of ligand bound to the receptors. They postulated that these contact sites could be targets for drug discovery. Analogous suggestions have previously been made for the use of corepressor or coactivator-occupied receptors to screen for new ligands [9]. The studies of Norris *et al* [12], along with those of others cited herein, indicate that we are at the brink of important insights into the molecular mechanisms by which ER and their ligands regulate hormone dependence and resistance in breast cancers. These insights will bring completely new approaches to treating these tumors, and if their promise is confirmed they will allow us to predict, and pehaps even prevent or reverse, development of resistance. It is an exciting time to be studying the roles of steroid hormones in breast cancer!

References

1. Cosman F, Lindsay R: **Selective estrogen receptor modulators: clinical spectrum.** *Endocr Rev* 1999, **20**:418–434.

2. Jordan VC, Morrow M: **Tamoxifen, raloxifene, and the prevention of breast cancer.** *Endocr Rev* 1999, **20**:253–278.
3. Osborne CK: **Tamoxifen in the treatment of breast cancer.** *N Engl J Med* 1998, **339**:1609–1618.
4. Fornander T, Rutqvist LE, Cedermark B, *et al*: **Adjuvant tamoxifen in early breast cancer: occurence of new primary cancers.** *Lancet* 1989, **i**:117–120.
5. Howell A, Downey S, Anderson E: **New endocrine therapies for breast cancer.** *Eur J Cancer* 1996, **32A**:576–588.
6. Encarnacion CA, Ciocca DR, McGuire WL, *et al*: **Measurement of steroid hormone receptors in breast cancer patients on tamoxifen.** *Breast Cancer Res Treat* 1993, **26**:237–246.
7. Belani CP, Pearl P, Whitley NO, *et al*: **Tamoxifen withdrawal response.** *Arch Intern Med* 1989, **149**:449–450.
8. Horwitz KB. **Editorial: when tamoxifen turns bad.** *Endocrinology* 1995, **136**:821–823.
9. Jackson TA, Richer JK, Bain DL, *et al*: **The partial agonist activity of antagonist-occupied receptors is controlled by a novel hinge-domain binding coactivator, L7/SPA and the corepressors N-CoR and SMRT.** *Mol Endocrinol* 1997, **11**:693–705.
10. Horwitz KB, Jackson TA, Bain DL, *et al*: **Nuclear receptor coactivators and corepressors.** *Mol Endocrinol* 1996, **10**:1167–1177.
11. McKenna NJ, Lanz RB, O'Malley BW: **Nuclear receptor coregulators: cellular and molecular biology.** *Endocr Rev* 1999, **20**:321–344.
12. Norris JD, Paige LA, Christensen DJ, *et al*: **Peptide antagonists of the human estrogen receptor.** *Science* 1999, **285**:744–746.
13. Norman TC, Smith DL, Sorger PK, *et al*: **Genetic selection of peptide inhibitors of biological pathways.** *Science* 1999, **285**:591–595.

Author address: Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado, USA

Correspondence: Kathryn B Horwitz, Department of Medicine, University of Colorado Health Sciences Center, Box B151, 4200 East Ninth Avenue, Denver, CO 80262, USA