Structural insights into the mechanisms of agonism and antagonism in oestrogen receptor isoforms

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Abstract

Here we summarise the results that have emerged from our structural studies on the oestrogen receptor (ER) ligand-binding domain. We have investigated the conformational effects of a variety of ligands on the structures of both ER isoforms. Each class of ligand (agonists, partial agonists and selective oestrogen receptor modulators) induces a unique conformation in the receptor's ligand-dependent transcriptional activation function. Together these studies have broadened our understanding of ER function by providing a unique insight into ER's ligand specificity and the structural changes that underlie receptor agonism and antagonism.

Keywords: Agonist; Antagonist; Crystal structure; ERα; ERβ; Transactivation

Oestrogens play a critical role in the growth, development and maintenance of a diverse range of tissues. They exert their physiological effects via the ER, which functions as a ligand-activated transcription factor. Both ER isoforms (ERα and ERβ) exhibit the characteristic domain organisation of eukaryotic nuclear receptor (NR) transcription factors that include a variable N-terminal transactivation domain, a highly-conserved DNA binding module and a carboxy terminal ligand-binding domain (LBD). The LBD is multi-functional and, in addition to harbouring a ligand recognition site, contains regions for receptor dimerisation and ligand-dependent (AF2) transactivation. The precise mechanism by which ER affects gene transcription is poorly understood but, at least in the case of AF2 activation, appears to be mediated by a host of nuclear factors that are recruited by the DNA-bound receptor [1].

Studies on the three-dimensional structures of NR-LBDs have provided a wealth of information that has enriched our view of these ligand-activated transcription factors. We have determined the structure of human ERα-LBD in complex with its endogenous hormone, 17β-oestradiol (E_2) and with the selective oestrogen receptor modulator (SERM) raloxifene (RAL; Evista™) [2]. More recently, we have also determined the structure of human ERβ-LBD in the presence of the phyto-oestrogen genistein (GEN) and RAL [3].

ER-LBD recognises a wide variety of structurally distinct compounds that act as either receptor agonists, antagonists or have mixed character. All ligands bind in a hydrophobic cavity within the core of the receptor. Recognition is achieved through a combination of specific hydrogen bonds and the complementarity of the hydrophobic residues that line the cavity to the non-polar nature of ER ligands. The ability of ER to bind a wide repertoire of compounds stems from both the size and shape of the hormone binding cavity which permits a variety of ligand binding modes [2,3].

While the overall conformation of the ER-LBD is remarkably similar in the various ligand complexes, one aspect of the LBD, namely the orientation of the C-terminal transactivation helix (H12), is highly sensitive to the nature of the bound ligand. The ability of ER ligands to act as agonists or antagonists can be related to the position adopted by this carboxy terminal helix (H12). This region of ER contains residues that have been shown to be important for the receptor's ability to activate gene transcription in a ligand-dependent manner. In the presence of agonists, H12 is orientated across the cavity so that the bound ligand is completely buried within the core of the LBD. In contrast, the large side-chain substituent of RAL, which is a characteristic feature of ER AF2 antagonists, can not be accommodated within the confines of the binding cavity. Instead, the substituent protrudes from the binding cavity and

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prevents the correct alignment of H12. Based on these observations, we proposed a structural model for RAL antagonism in which H12, the transactivation helix, is repositioned on SERM binding, thereby disrupting ER's interaction with coactivators in some way [2]. Subsequent studies have shown that the position adopted by H12 in the presence of agonists results in the formation of a specific recruitment surface for essential NR coactivators [4]. The antagonistic effects of SERMs, therefore, appear to arise from a ligand-induced, suboptimal conformation of ER that is unable to communicate with the cellular transcriptional machinery.

The next challenge for structural biology is to determine the structures of full-length receptors in complex with DNA and/or coactivators and corepressors. Such structures should provide additional insight into the mechanism of action of these important receptors.

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Detection of the oestrogen receptor (ER): immunohistochemical versus cytosol measurements

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Abstract

Oestrogen receptor (ER) content gives a direct indication of the chances that a breast cancer patient will show a sustained response to endocrine therapy. Thus, an ER value should be recorded for every breast cancer patient. ER was traditionally measured by a ligand binding assay (LBA). LBA is not suitable for all routine hospitals in which breast cancer is treated. More appropriate is immunohistochemistry (IHC). This paper identifies advantages and disadvantages of both assays, suggests that both methods predict equally response to endocrine therapies and describes a simple, semi-quantitative IHC for which external quality assurance works successfully.

Keywords: Oestrogen receptor; Breast cancer; Therapy; Assay protocol; Immunohistochemistry

1. Introduction

The overview of early breast cancer treatment [1] has shown that benefit from endocrine therapy is directly proportional to the amount of ER present in the tumour. Thus, ER information should be available for all breast cancer patients. However, the ER information used in the overview was obtained almost exclusively by the LBA. For various reasons, the LBA is being rapidly displaced by the IHC assay. In these days of evidence-based medicine, it would be inappropriate to switch from one assay to another without checking the relevant accuracy and reproducibility of the two assays in terms of both numbers and predictive accuracy.

2. Requirements of an assay for ER

Experience [2] has led to the view that the minimum requirements for any receptor assay are that it should be quantative, specific, be appropriate for the tissue, measure functional protein and be clinically predictive. Early attempts to meet some or all of these criteria

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