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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involved assays based on sucrose density gradients, gel filtration electrophoresis, saturation and, more recently, immunology. Most of the early techniques were discarded because they were either very time-consuming or they failed external quality assurance (EQA) tests in multi-laboratory studies. The assay adopted universally was the multi-point saturation assay using Scatchard analysis. This was successfully subject to EQA and supply of external standards led to good correlation, at least among 'expert' laboratories [3].

The advantages of the ligand binding assay [4] include the generation of numerical results across the whole of the likely concentration range, good reproducibility, full technical and clinical validation, inclusion of measures of receptor functionality (such as activation of the progesterone receptor gene) and existing quality assurance schemes. Disadvantages include the relatively large amounts of tissue required, the necessary care over handling, storage, assay and data processing, the labour intensive nature of the assay, and the lack of information about the nature of the tissue being homogenised.

These disadvantages led to the view that the LBA would not be suitable for introduction into routine laboratories. The introduction of population screening had also led to a marked reduction in the size of biopsy available for assay. As improved anti-ER antibodies became available, the IHC assay began to replace the LBA. However, there were again advantages and disadvantages of the IHC assay. Advantages included the fact that routine, fixed material could be used; archival material could be assayed retrospectively; only small quantities of tissue (including fine needle aspirations and core biospies) were needed; receptor content could be related to morphology and there was a measure of cellularity; internal positive control was often provided by the normal epithelial tissue in the section. The disadvantages included the subjectivity; the lack of quantitation; the absence of any indication of functionality of the receptor; the lack of standardisation of staining; the absence of an appropriate QA scheme and the lack of clinical validation.

Many of these disadvantages are eliminated by adopting a common protocol. One being used successfully [6] involves adequate fixation, the incorporation of normal parenchyma, rules on localisation, pre-coated slides, defined antibody retrieval, use of an established antibody and avoidance of surfactants. Incorporation of ABC signal ampification, careful counterstaining, measurement of nuclear staining only and regular inclusion of EQA slides has led to some highly reproducible results. An 8-point scoring system awards from 0–5 points according to the proportion of nuclei staining and 0–3 for the intensity of staining, with corresponding clinical recommendations, according to the final score. Using this scoring system, Harvey and colleagues [5] have shown that, although LBA was not significant in predicting disease-free survival, IHC was. Both methods predicted strongly and equally well for overall survival (almost all ER-positive patients receiving tamoxifen) and the authors concluded that IHC was probably the more clinically useful tool. In a UK study, Barnes and colleagues [4] have also shown that IHC, again using this simple scoring system, has very good predictivity in relation to response to endocrine therapy and overall survival.

There is still some debate about whether IHC should have a clinical cut-off point since raising the cut-off demonstrably raises specificity, but lowers sensitivity [4].

3. Conclusions

The immunohistochemical assay is now established as at least as good in terms of clinical prediction of response to endocrine therapy as is the ligand binding assay. There is a simple scoring system which gives sufficient quantitative information to allow flexible clinical recommendations as well as allowing successful external quality control. ER (and, probably, progesterone receptor status) should be determined semi-quantitively (within an EQA programme) for all new breast cancer patients.

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