E5. Molecular basis of endocrine therapy

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The steroid hormone oestradiol is of pre-eminent importance in the development and growth of most breast carcinomas and is a target for their hormonal therapy. Such treatment is based on either the blocking of the stimulatory effects of oestrogens or the withdrawal of oestrogens by suppressing their synthesis. Over recent years, pre-clinical and clinical studies of the molecular interactions between oestrogens and their cognate binding protein, the oestrogen receptor (ER), have revealed a high level of complexity that may influence the response of individual lesions to particular therapeutic modalities. A full understanding of these interactions is necessary for a rational approach to hormonal therapy and for the application of new targeted agents alongside this therapy.

Oestrogens are synthesised by the action of the aromatase enzyme from androgenic substrates (testosterone being converted to oestradiol and androstenedione to oestrone). In premenopausal women, the richest source of aromatase is in the granulosa cells of the ovarian follicle where it is subject to feedback control by gonadotrophins. In postmenopausal women, the ovaries are devoid of aromatase, but residual, much lower levels of oestrogen persist as a result of the presence of aromatase in peripheral tissues, particularly subcutaneous fat and normal breast cells.

Oestrogens pass across the cell membrane by free diffusion and bind to the ER, almost exclusively as oestradiol, in the nucleus of oestrogen-sensitive cells. Two species of ER may be present, ERα and ERβ, which are the product of individual genes. At present, no clear role for ERβ has been defined in breast cancer and the comments on ER discussed below apply exclusively to ERα.

ER that is unbound to ligand exists in a monomer form, but binding to ligand elicits a series of molecular changes that include a conformational change, phosphorylation on a series of amino acid residues throughout the molecule and dimerisation. These processes result in the activation of two transcriptional activating functions (AF1 and AF2). The activated dimer binds to specific nucleotide recognition sequences in DNA called oestrogen response elements (EREs) that are found upstream of oestrogen-responsive genes. Further proteins called co-activators are recruited to the ER-DNA complex and these lead to acetylation of the surrounding histone and stablisation of the basic transcriptional machinery, thereby propagating transcription of the gene. Recent evidence indicates that this transcriptional activity also leads to the ubiquitination of the ER and this directs the protein to proteasomal degradation. The mechanism is thus set to respond rapidly to changes in the ambient level of oestrogens [1].

While it seems clear that the bulk of ER exists in cell nuclei, there is increasing evidence that some ER may exist in the cell membrane where it can interact directly with growth factor receptors and other signalling proteins to produce a number of non-genomic effects. The potential clinical importance of this membrane-bound ER is not established and may be difficult to study with the currently available diagnostic reagents.

ER is present in approximately 75–80% of breast carcinomas with the proportion of positivity increasing with age. Those tumours that are ER-positive are targets for endocrine therapy. Other than the small proportion of ER-negative tumours that are PgR-positive, tumours lacking measurable ER are non-responsive to oestrogen therapy.

Tamoxifen remains the most widely used endocrine agent for breast cancer therapy and is effective in pre- and post-menopausal patients. It works by binding to the ER and blocking the binding of oestrogen. However, on binding the ER tamoxifen elicits a relatively similar cascade of events to oestrogen including dimerisation, phosphorylation and a conformational change, but the latter is different to that caused by oestrogen [2,3]. The result is that AF1, but not AF2, is activated. Transcription of a particular gene may or may not occur according to the presence of co-activators that may or may not bind to the tamoxifen-bound ER. Thus, antagonism of certain genes, but a degree of agonism of others occurs. So far as breast cancer is concerned, the overall effect is predominantly antagonistic, but the partial agonism impedes this leading to incomplete oestrogen antagonism. Greater degrees of agonism occur on the bone, which is advantageous in postmenopausal women, but also on the endometrium and this leads to an enhanced risk of endometrial cancer.

Over recent years, because of this differential antagonism of different oestrogen responsive tissues, tamoxifen has become known as a selective oestrogen receptor modulator (SERM) and a series of new SERMs have been developed with reduced agonism on the breast and

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endometrium [4]. However, the clinical outcome of the use of these agents has generally been disappointing, with none showing a substantially greater efficacy than tamoxifen or significant activity in tamoxifen-resistant tissues, although raloxifene remains of interest in the area of breast cancer prevention. In contrast, the pure steroidal anti-oestrogen, fulvestrant, shows substantial activity in tamoxifen-resistant patients, possibly because of its different mode of action which involves no activation of the ER and shortening of the protein's half-life which results in markedly lower levels of the ER being available for signalling.

Oestrogen deprivation is achieved differently in pre- and post-menopausal women. In pre-menopausal women, ovarian oestrogen production is the target and, in recent years, this has commonly been attacked using gonadotrophin-releasing hormone (GnRH) agonists, such as goserelin and leuprorelin. These act by downregulating GnRH receptors on the pituitary gonadotrophs leading to the suppression of gonadotrophins from them and therefore near complete ablation of ovarian function.

In postmenopausal women, modern aromatase inhibitors lead to profound inhibition of aromatase in peripheral tissues and suppression of plasma oestrogen levels to below the detection limit of our most sensitive assays.

The molecular impact of such substantial oestrogen deprivation is to markedly reduce ER activation. Since these agents do not bind to the receptor, there is no possibility of the deprivation being attenuated by partial agonist activity (as with tamoxifen). Trials of aromatase inhibitors versus tamoxifen in advanced and early disease indicate that this is an important difference since in each case the aromatase inhibitor has been more effective than tamoxifen [5]. In the ATAC trial of adjuvant hormonal therapy the combination of tamoxifen with the aromatase inhibitor, anastrozole, showed similar efficacy to tamoxifen alone and less than the aromatase inhibitor alone [6]. This indicated that the agonist activity of tamoxifen was dominant in this combination and suggested that there would be no therapeutic advantage of combining a SERM with the agonist activity of tamoxifen with an aromatase inhibitor.

Recent preclinical evidence indicates that there is major cross-talk between growth factor receptors and ER signalling pathways [7,8]. For example, forced overexpression of HER2 leads to increased levels of ER phosphorylation and sensitisation to the agonist effects of tamoxifen. These data are paralleled by a series of trials suggesting that tamoxifen is less effective in ER+HER2+ patients and, most recently, by data indicating that the effects of aromatase inhibitors may be more efficacious in such tumours. Further work that confirms that these pathways are of clinical significance will provide the evidence needed to select the most appropriate drugs for endocrine treatment according to the molecular features of a patient's tumour. This should also enable us to direct new agents that are targeted at signal transduction pathways in an appropriate manner in combination with endocrine therapy.

References

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