ESTROGEN AND PROGESTERONE RECEPTOR ANALYSIS AND ACTION IN BREAST CANCER

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INTRODUCTION

The elucidation of the molecular mechanisms responsible for the hormonal regulation of cell proliferation in breast cancer has been the object of intense research. Because most breast cancers are initially dependent upon estrogens for continued growth, much of this research has focused on the role of estrogen receptor (ER) in the control of gene expression and mitosis, and on its use as marker for hormone responsiveness and prognosis. In addition, progesterone receptor (PR), as both a mediator of hormonal responses and as a product of estrogen action on breast cancer cells, has been studied extensively as a tumor marker and in terms of its regulation by estrogen agonists and antagonists. Although its function in breast cancer is unknown, the presence as well as the induction of PR has been coupled to estrogen-induced proliferative responses in breast cancer cells. An improved understanding of the function and regulation of expression of these transcription factors is emerging from studies of the structure, composition and dynamics of the receptor proteins and the genes that encode them. The recent cloning and molecular analysis of all of the known steroid receptors has led to the definition of common functional domains and a proposed mechanism by which they interact with responsive genes, via cis-acting DNA enhancer elements, in normal and neoplastic tissues. For ER and PR, these studies have been aided by the availability of a number of monoclonal antibody probes directed against specific regions of each receptor. In addition, the same antibodies have been used to develop validated quantitative and histochemical immunoassays for ER and PR in a variety of hormone-responsive tissues and related cancers. These assays have proved particularly useful in the evaluation of ER and PR in breast tumor extracts, in frozen and paraffin-embedded tumor sections, and in needle biopsies. The focus of this paper is on the current knowledge of ER and PR structure, composition and dynamics in breast cancer cells as a function of agonist and antagonist binding.

THE STEROID RECEPTOR FAMILY

The estrogen and progesterone receptors, like all of the steroid receptors, are members of a large family of trans-activating transcription factors that are activated by a ligand and bind with high affinity and specificity to short DNA enhancer elements called hormone response elements (HREs). Interaction of steroid-receptor complexes with responsive genes in vivo can result in either up or down regulation of transcription, depending upon the target gene and the tissue. The molecular mechanisms by which either pathway occurs are still
obscure, although it is generally believed that receptor-DNA complexes recruit, or allow the recruitment of, other transcription factors that comprise a functional transcription complex. This process might involve protein-protein interactions between receptor and other factors, resulting in the formation of DNA loops to accommodate long stretches of DNA between promoters and HREs, or possibly by altering the local chromatin organization to permit access of other transcription factors; obviously, both events could occur. Although it is widely believed that an allosteric alteration of receptor structure occurs following hormone binding, exposing the DNA-binding domain, the nature of this change is not understood. The participation of other proteins, such as the heat shock protein hsp90, may be important in stabilizing the inactive form of receptor, as has been suggested for glucocorticoid receptor. However, to date, despite extensive in vitro data that demonstrates the association of several members of this family with hsp90 in cell free systems, there is no in vivo evidence to link the hormone-binding receptor protein with hsp90 or any other protein prior to ligand-induced activation. It has also been suggested that nonhistone protein acceptor sites (part of nuclear matrix?) play a key role in receptor action, possibly by directing receptor to a target gene. Although such sites have been described, they have not yet been linked in an obligatory manner to a functional transcription complex. Another unresolved issue is the role of phosphorylation in receptor function or dynamics. At least two levels of phosphorylation have been described. For estrogen receptor, and possibly for glucocorticoid receptor, it appears that phosphorylation on tyrosine may be required to activate hormone binding. However, this observation remains controversial. A second level of phosphorylation would be the hormone-induced phosphorylation on multiple serine residues that has been described for rabbit, chicken and human PR and for vitamin D receptor and glucocorticoid receptor. At this point, no functional role for this phosphorylation has been defined. However, it does not appear to influence receptor activation or binding to DNA or, for PR, processing of receptor, although an effect on GR recycling has been proposed. It is more likely that phosphorylation may be affecting interaction of these receptors with the transcriptional machinery. Finally, the nature of agonist- vs antagonist-receptor interaction is poorly understood at present. It seems likely that an altered conformation of receptor occurs in the presence of an antagonist, which could affect DNA binding, interaction with other transcription factors, phosphorylation, or interaction with hsp90. At least for PR, the pattern of phosphorylation appears to be the same in T47D human breast cancer cells when cells are exposed to either a progestin or antagonist, although the level of phosphorylation is higher in the presence of antagonist, suggesting that phosphorylation may be sensitive to agonist/antagonist differences. Obviously, there are still a number of key dynamic and molecular aspects of receptor activity that are not resolved at this time.

STRUCTURE AND PROPERTIES OF HUMAN ER

The isolation, sequencing and expression of a 2.1 kb cDNA containing all of the translated sequence for ER mRNA from MCF-7 human breast cancer cells has provided a wealth of information about the composition and functional domains of ER. An open reading frame of 1785 nucleotides encodes a protein of 595 amino acids with Mr 66,200. This ORF is contained in a 6.6 kb mRNA that includes 4.3 kb of 3' untranslated sequence and approximately 230 nucleotides of 5' untranslated sequence. The long untranslated 3' sequence is characteristic of all of the steroid receptors and its function is unknown, although HREs have been found in this region of the rat glucocorticoid receptor mRNA. A schematic representation of the human ER protein is shown in Fig. 1. The positions of all proline, cysteine, and basic (lys/arg) residues are indicated. It is noteworthy that a significant proportion of the prolines are in the amino terminal portion of the protein, as is also the case for PR. The consequences of this clustering of prolines are not yet known. However, the secondary structure of this portion of the receptor must be considerably altered from an a-helix in this region. This is also the region of greatest immunogenicity for PR and GR, but not ER, which may be due in part to its structural organization, as well as to a generally lower sequence homology in this region among receptors from different species. A comparison of