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Estrogen receptor β in the breast: role in estrogen responsiveness and development of breast cancer

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Abstract

Breast cancer is one of the most common forms of cancer observed in women. Endogenous estrogen is thought to play a major role in its development and estrogen receptor blockers are the most important drugs in its treatment. It has long been thought that any conditions or exposures, which enhance estrogenic responses, would result in an increased risk for breast cancer. The discovery of the second estrogen receptor, ERβ, which can have effects opposite to those of the well-known 'original' estrogen receptor (now called ERα) challenges this simplistic view. In order to understand breast cancer one must first understand how the normal breast is maintained. The functions of ERβ in the breast remain to be defined but from what we have learnt about its activities in in vitro systems, this estrogen receptor may have a protective role in the breast. Studies in human and rodent breasts as well as in human breast cancer biopsies reveal that ERβ is by far the more abundant of the two ERs. Despite the role of estrogen in proliferation of the breast, neither of the two ERs appears to located in epithelial cells which divide in response to estrogen. In order to define the functions of ERβ in the normal and malignant breast, we have created mice in which the ERβ gene has been inactivated. Studies of the breasts of ERβ knock out mice (BERKO) revealed abnormal epithelial growth, overexpression of Ki67 and severe cystic breast disease as mice age. © 2000 Published by Elsevier Science Ltd.

Keywords: Breast cancer; Estrogen; Ligand-binding domain

1. Introduction

1.1. Background

Estrogen is a modulator of cellular growth and differentiation. Its major targets are the mammary gland, uterus, bone, cardiovascular system, brain and urogenital tract of both males and females [1–4]. Estrogen mediates its functions through two specific intracellular receptors, ERα and ERβ, which act as hormone-dependent transcriptional regulators [5]. The biological importance of estrogen is evident from the number of disease states associated with altered production of estrogen or abnormalities in the estrogen receptor. Osteoporosis, breast cancer, endometrial cancer and prostate hypertrophy are some of the disorders in which estrogen receptors are involved. As with all other signaling molecules in the body, which stimulate growth, estrogen is involved in carcinogenesis.

1.2. Estrogen and breast cancer

Since it is essential for growth and development of the mammary gland, estrogen has been associated with promotion and growth of breast cancer. Numerous animal studies show that estrogen can induce and promote breast cancer, and removal of the ovaries or administration of antiestrogens can oppose this [6–9]. Most human breast cancers, at least initially, are hormone-dependent and they undergo regression when deprived of the supporting hormone. The presence of significant amounts of ERα in breast cancer at the time of diagnosis is generally taken as an indication of hormone dependence [10], and, on this basis, treatment with antiestrogen (tamoxifen) is now the first-line therapy for metastatic diseases [11]. About two-thirds of patients with ERα-positive breast tumors will respond favorably to tamoxifen treatment or other endocrine manipulations. Despite initial benefits, most patients on

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tamoxifen therapy eventually relapse with tumors which not only have become tamoxifen-resistant but which actually are stimulated by the medication. Current immunochemical procedures for ER measurements are based on ERα protein and do not detect ERβ protein. The role of ERβ in determination of the outcome of endocrine ablation has, therefore, been completely overlooked.

1.3. ERα and ERβ

ERα and ERβ are similar in their architecture to the other members of the steroid/thyroid hormone superfamily of nuclear receptors [12–14] in that they are composed of independent but interacting functional domains. With nuclear receptors, binding of the cognate ligand causes conformational changes in the receptor which elicit other events including receptor dimerization, receptor:DNA interaction, recruitment of and interaction with co-activators and other transcription factors, and the formation of a preinitiation complex. The N-terminal A/B domain of ER encodes the ligand-independent activation function 1 (AF1), a region of the receptor involved in protein:protein interactions and transcriptional activation of target gene expression.

The C- or DNA binding domain (DBD) contains a two zinc finger structure, which plays an important role in DNA sequence-specific receptor binding and receptor dimerization. The carboxy-terminal E/F- or ligand-binding domain (LBD) mediates ligand binding, receptor dimerization, nuclear translocation, and transactivation of target gene expression. Activation function 2 (AF2) in the LBD constitutes the ligand-dependent transcription activation function of nuclear receptors.

1.4. ERβ variants

Several splice variants of ERβ have been described. Some have extended N-termini and others have truncations and/or insertions in the LBD. A human ERβ cDNA encoding a protein of 530 amino acids, due to an N-terminal extension, composed of 45 amino acids, was identified in 1998 [15]. Later, a rodent ERβ isoform with 64 extra amino acid residues compared with the original rat ERβ clone (ERβ-485) was reported [16]. In addition to extensions of the N-terminus, three groups have reported the cloning of a 503 amino acid long ERβ isoform with an 18 amino acid residue in-frame insertion into the LBD [17–19], in the splice junction between exon 5 and 6 [20].

In contrast to the N-terminally extended ERβ isoforms the affinity of ERβ-503 for E2 and other known estrogen receptor ligands is 10^2–10^3-fold lower than that for ERα and ERβ-485. ERβ-503 acts as a dominant negative regulator of estrogen action by suppressing the estrogen-dependent ERα and ERβ-mediated activation of gene transcription [19,21]. Both ERβ-503 and ERβ-485/530 bind to a consensus estrogen response element (ERE) and heterodimerize with each other and with ERα [18,19].

ERβcx [22] is identical to ERβ-530 except that the last 61 C-terminal amino acids have been replaced by 26 unique amino acid residues. Due to the exchange of the last exon, ERβcx lacks amino acid residues important for ligand binding and for the core of the activation function 2 (AF2), which is important for ligand-dependent receptor:coactivator interactions. It is, therefore, not surprising that ERβcx shows no ligand binding activity and has no capacity to activate transcription of estrogen-sensitive reporter genes. ERβcx heterodimerizes preferentially with ERα. Functionally, the heterodimerization of ERβcx with ERα has a dominant negative effect on ligand-dependent ERα reporter-gene transactivation.

Because ERα-containing epithelial cells in the normal breast do not proliferate in response to estrogen [23–25], the mechanisms through which estrogen induces epithelial growth in the breast are not clear. The prevailing concept is that estrogen stimulates secretion of growth factors from breast stroma and that these factors stimulate epithelial cells to proliferate [26]. This explanation leaves two questions: (1) why do ERα-containing normal cells not proliferate? and (2) why does breast cancer epithelium, which contains ERα, proliferate in response to estrogen? One possible explanation of estrogen action on epithelial cells is that ERβ-containing cells are the ones which proliferate. We have tried over the past 2 years to approach these questions by studying both the rodent and human breast.

2. Results and discussion

2.1. Results from our rodent mammary gland studies

In the rodent breast ERβ is constitutively expressed in approximately 70% of epithelial cells, regardless of the endocrine state of the breast [27]. In virgin, pregnant and post lactation breast, few cells express ERα. ERβ is highly expressed during lactation when it is co-expressed with ERα in over 70% of epithelial cells. During the highest proliferative phase of the breast, i.e. pregnancy, there is very little expression of ERα, high expression of ERβ, but most of the cells which express the proliferating cell antigen contain neither receptor. These results suggested two possibilities to us: (1) the presence of estrogen receptors in epithelial cells prevent these cells from proliferating or (2) the effects of estrogen on the breast are indirect, most likely via the immune system.

In order to test these ideas, new approaches to the study of the role of estrogen in proliferation of breast epithelium are required. We hypothesize that estrogen receptors down regulate growth factor receptors in epithelial cells. Malignancy would then be a state where growth factor receptors have escaped from this negative regulation by estradiol. In support of this idea, studies show that, in sublines of breast cancer cells in culture, loss of ERα is accompanied by an increase in growth factors and growth factor receptors, and in higher levels of phosphotyrosine residues indicating an increased tyrosine kinase activity [28]. Growth factors and their receptors have been extensively studied in the mammary gland [29]. We hypothesize that release of growth factors from the stroma is indirect via the release of cytokines from the immune system.

2.2. Results from our human breast study

In the past year there have been several publications showing that ERβ is expressed in human breast cancer [30–32] and conclusions and speculations about a causative role for ERβ in breast cancer development and/or progression have been made.

We have begun a large study with 500 frozen breast biopsies in collaboration with Professor R.C. Coombes, Director of Cancer Research Campaign Laboratories, Department of Cancer Medicine, CRC Laboratories, Hammersmith Hospital Campus, in order to clarify the role of ERβ in normal and malignant breast. The first part of this study (30 samples) has been completed. In this study we measured ERα and ERβ proteins by several techniques (immunohistochemistry, Western blotting, ligand binding in sucrose gradients, and RT-PCR) in various human samples obtained from both benign and malignant breast. We found that ERβ is the predominant estrogen receptor in the normal mammary gland and in benign breast disease. There is very little ERα in the normal mammary gland. ERα is abundantly expressed in invasive and in situ ductal carcinoma but not in medullary cancer. ERβ is also expressed in breast cancer, both ductal and medullary. We, therefore, could conclude that the presence of ERβ as such is not a marker nor is it of any prognostic value in breast cancer. What was more important was the finding of ERβcx in breast cancer. Samples which had ERβcx had no estrogen binding in the ERβ peak on sucrose gradients although there was a clear ERβ signal on Western blots. This was the first demonstration that ERβcx is expressed as a protein. This discovery has set in motion a new set of experiments. If ERβcx inhibits ERα action, its presence in ERα containing cells may result in expression of growth factor receptors on these cells and provide a solution to the dilemma of why ERα containing cells can proliferate in cancer but not in normal mammary glands.

There were two other surprising findings in this study which have necessitated several new lines of investigation about the function of ERβ in the breast. These are:

1. The major ER in breast stroma is ERβ. This is surprising because it has long been thought that ERα in the stroma was responsible for secretion of growth factors in response to estradiol. The discovery that it is ERβ in the stroma suggests a new series of experiments to investigate the role of ERβ in growth factor secretion.
2. Many postmenopausal women have activated ERα in the nuclei of their breasts suggesting activation of ER independent of estrogen. This observation has also pointed to a new direction, i.e. a study of phosphorylation of ERα and ERβ in breast cancer and the measurement of ER ligands in human breast issues.

2.3. Our observations on BERKO breasts

The breast develops rather normally in BERKO mice with the ducts extending to fill the fat pad. This is unlike the ERα knock out mice where there is very little ductal growth. After puberty, the breasts of BERKO mice do not undergo cyclical changes since very few corpora lutea are formed in the ovary and progesterone secretion is limited. BERKO breasts do respond to administered progesterone with increased ductal branching. At 2 years of age BERKO mice, but not their wild type littermates, develop severe cystic breast disease which appears similar to postmenopausal mastopathy, and, in addition, the breast epithelium becomes disorganized and the epithelial cells express Ki67 indicating that they are not in Go.

Mastopathy occurs in both young and old women but it is very common in postmenopausal women. The main symptom is painful breasts and there is no treatment. Seventy percent of all mastopathies do not exhibit proliferation, but approximately 10% show an atypical proliferation that suggests an increased risk for cancer development [33]. Some patients with mastopathy have high levels of estradiol and low natural killer cell activity, a situation which is considered an increased risk for neoplasia [34].

3. Significance

Study of ERβ in the breast has relevance to our understanding of the mechanisms of estrogen action in general. It is also essential for an understanding of breast cancer and its treatment and in addition, it provides an opportunity for development of new drugs with selective estrogenic actions. A better understanding how estrogen mediates its proliferative effects in breast cancer cells will give rise to better tools for monitoring hormone-dependent tumor growth and improved methods for treatment of the disease.

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