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Review

Estrogen receptor beta in breast cancer

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ABSTRACT

Estrogen is essential for growth and development of the mammary glands and has been associated with the promotion and growth of breast cancer and in line with this, most human breast cancers are initially estrogen-dependent and undergo regression when deprived of their supporting hormone. Estrogen exerts many of its effects via two nuclear estrogen receptors (ERs), ERα and ERβ. The discovery of a second ER, ERβ, demanded a full re-evaluation of estrogen action in all target tissues and different estrogen associated diseases, including human breast cancer. However, despite over 15 years of research, the exact role, if any, of ERβ in human breast cancer remains elusive. The main challenges now are to develop highly selective anti-ERβ antibodies that are applied to large well characterized human breast cancer samples to validate their diagnostic potential and to explore ERβ-selective agonists in animal models of breast cancer to validate their therapeutic potential.

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The discovery of a second estrogen receptor (ER), ERβ, demanded a full re-evaluation of estrogen action in all target tissues and different estrogen associated diseases, including human breast cancer. However, despite over 15 years of research, the exact role, if any, of ERβ in human breast cancer remains elusive and today only ERα is used in making clinical decisions and as the targeted receptor.

This review aims to review our current understanding of ERβ in relation to breast cancer, what has been learnt from studying *in vitro* cellular models, animal models as well as clinical samples and human genetics. It is clear that ERβ should be explored for its potential to stratify breast cancer diagnosis and as a target for novel therapeutic avenues particularly as only 70% of ERα positive cases respond to tamoxifen. We suggest that challenges now are to develop highly selective anti-ERβ antibodies that are applied to large well characterized human breast cancer samples to validate their diagnostic potential and to explore ERβ-selective agonists in animal models of breast cancer to validate their therapeutic potential.

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1. Introduction

Breast cancer is the second most common cancer and the most common cancer among women in the world (Parkin et al., 2005). It is a heterogeneous disease that includes several distinct subtypes with distinctive gene expression patterns and different overall survival (Sorlie et al., 2001). The treatment of breast cancer has been greatly advanced in the past decades due to the discovery of specific predictive and prognostic biomarkers that enable the application of more individualized therapies to different molecular subgroups with distinct clinical behavior (Sawyers, 2008).

Studies of breast cancers using gene expression profiling have identified several major molecular subtypes, including luminal A, luminal B, human epidermal growth factor receptor 2 (HER2) (also known as ERBB2)-overexpressing, normal breast tissue-like, and basal-like (Sorlie et al., 2001, 2003). Based on immunohistochemical staining of ERα, progesterone receptor (PR) and HER2, breast cancer falls into the following subgroups;

Luminal, subtype A: ERα and PR positive, HER-2 negative, luminal, subtype B: ERα, PR and HER-2 positive, HER2 overexpression: ERα and PR negative, HER-2 positive and triple-negative breast cancer (TNBC): ERα, PR and HER-2 negative.

2. Estrogen signaling in the normal breast and breast cancer

Estrogen is essential for growth and development of the mammary glands and has been associated with the promotion and growth of breast cancer. In normal mammary tissue, ERβ is the most widely expressed ER, and is expressed in both luminal and myoepithelial cells as well as in some cells in the surrounding stroma (Speirs et al., 2002).

Studies of ERβ knock-out (KO) (ERβKO) mice suggest a minor effect of ERβ on the mammary gland as ERβKO animals undergo an overall normal mammary gland development. Subtle effects associated with decreased differentiation and increased proliferation in the alveoli of lactating mammary glands are sometimes observed in these mice. These changes appear to be age related and are only observed in some ERβKO models (Forster et al., 2002; Palmieri et al., 2002).

Most human breast cancers are initially estrogen-dependent and undergo regression when deprived of their supporting hormone. The presence of significant amounts of ERα in breast cancer at the time of diagnosis is taken as an indication of hormone dependence (Huang et al., 2005). On this basis, treatment with ERα antagonistic compounds, such as tamoxifen, is first line for adjuvant therapy. Introduction of ER antagonists for treatment of hormone responsive breast cancer represents a milestone in the treatment of this life threatening disease. However, ERα status is not a perfect marker for responsiveness to antiestrogens; only 70% of ERα positive cases respond to tamoxifen. Importantly, 30–40% of patients receiving adjuvant tamoxifen therapy eventually relapse. Thus, resistance to endocrine therapy is a significant clinical problem. Clearly, additional and/or complementary factors are necessary to more accurately define patients who will benefit from the above therapy and to derive novel treatment strategies. One such factor could be ERβ that should be explored for its potential predictive value as well as a potential drug target.

3. Human ERβ

The human ERβ gene (*ESR2*) is located on chromosome 14q23.2, spanning ~61.2 kb. The ERβ protein is produced from eight exons. The full-length human ERβ (also named ERβ1) protein includes 530 amino acids with an estimated molecular mass of 59.2 kDa (Ogawa et al., 1998a).

The ER ligand-binding domain (LBD) is composed of 12 α helices. Agonist binding, in contrast to antagonist binding, stabilizes the overall conformation of the LBD and induces a conformation that promotes co-activator binding. The ligand-binding pocket of ERβ differs at only two amino acid positions to that of ERα. However, amino acid differences not directly part of the ligand-binding pocket as well as the overall smaller size of the ERβ pocket may contribute to the identification of compounds displaying receptor selectivity. Some ERβ selective ligands have been identified, one of the most studied is diarylpropionitrile (DPN). However, generally these ligands only display modest selectivity for ERβ versus ERα and there is clearly a need to develop novel compounds with improved selectivity.

Transcription of the human *ESR2* gene occurs from at least two different promoters, named promoter 0N and promoter 0K (Hirata et al., 2001). It should be noted that full-length ERβ cDNA sequences containing neither exon 0N nor exon 0K have been reported, suggesting the presence of (an) additional promoter(s). We have shown that transcripts from promoter 0N were more common than those from promoter 0K in normal breast epithelial cells and a panel of breast cancer cell lines (Zhao et al., 2003). Further studies aimed at characterization of promoters 0K and 0N and identification and characterization of putative additional promoters should aid in defining mechanisms for how expression of the human *ESR2* gene is regulated.

3.1. ERβ genetic variants

Many SNPs have been described for the *ESR2* gene. Importantly, none of these changes the amino acid sequence of the ERβ protein. Two commonly studied SNPs, rs4986938 and rs928554, are located in 3′ untranslated regions of *ESR2* and could affect mRNA stability and/or translatability. However, using allelic expression and luciferase assays we demonstrated that the two alleles of the respective SNPs were present at the same mRNA level and are expressed at equal protein levels in the context of luciferase constructs suggesting that the alleles of the two SNPs do not display differences with regard to mRNA stability and/or translatability (Putnik et al., 2009).

A number of association studies have been carried out to investigate the relationship between polymorphic sites in the *ESR2* gene and breast cancer risk. Overall, the results are inconclusive and few studies demonstrate significant association for a single allele. The lack of replication could reflect population based differences or limited cohort sizes. Yu et al. conducted a meta analysis for the most frequently studied SNPs, rs1256049 and rs4986938 and their association with breast cancer (Yu et al., 2011). Women harboring the variant allele rs4986938 seemed to present with decreased risk either in the dominant model or in the co-dominant model. rs1256049 was not associated with breast cancer risk in any model. However, five studies had investigated the effect of haplotypes in the *ESR2* gene on breast cancer risk, and four of them had positive outcomes. Furthermore, genome wide association studies (GWAS) have not provided evidence for an association between ESR2 and breast cancer.

In summary, there is presently no evidence that ESR2 is a major genetic determinant of breast cancer risk. However, the genetic contribution of ESR2 to breast cancer risk needs to be further explored. Particularly, a minor role of ESR2 variants in breast cancer or the role of these variants in linkage with other genes, particularly in certain types of breast cancer and in certain populations cannot be excluded. Future studies employing high throughput sequencing might reveal novel, less common, genetic variants in *ESR2* associated with risk of breast cancer.