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Estrogen and progesterone receptor isoforms: clinical significance in breast cancer

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Summary

The identification and exploitation of biomarkers that may predict response to anti-cancer treatments has the capacity to revolutionize the way that patients with cancer are treated. In breast cancer, the estrogen receptor (ER) and the progesterone receptor (PgR) are known to have a significant predictive value in determining sensitivity to endocrine therapies. Tumor expression of ER or PgR is known to affect clinical outcome and this information is often used to determine a patient's optimal treatment regimen. However, the measurement of ER and PgR alone is more complex than originally thought and the impact of the recently identified isoforms of ER (ERα and ERβ) and PgR (PgRA and PgRB), as well as several variant and mutant forms, upon the choice of treatment remains unclear. Therefore, ER and PgR expression alone are unlikely to determine a patient's optimal treatment regimen, particularly when the amount of 'cross-talk' between different pathways, such as the epidermal growth factor receptor pathway, is considered. In order to account for the complex cell-signaling environment that occurs in breast cancer, multifactorial techniques are needed to analyze tumor biomarker expression. The recent advances in genomic- or proteomic-based approaches has enabled molecular portraits of breast cancers to be painted, allowing biomarkers of response and prognosis to be identified and characterized more accurately than before. In the future, patients could be treated according to the molecular portrait of their tumor biomarker expression, maximizing the therapeutic benefit that each patient receives.

Predicting clinical response to anti-cancer therapies

In breast cancer, an increasing number of putative biomarkers that may prove to be significant in predicting clinical response to anti-cancer therapies are being identified. To exploit the utility of these new potential biomarkers, it is useful to first examine the most recent data surrounding the estrogen receptor (ER) and the progesterone receptor (PgR) in breast cancer, as both receptors are known to have a significant predictive value in determining sensitivity to endocrine therapies. Indeed, clinical practice guidelines indicate that patients with ER and/or PgR-positive disease should receive endocrine therapy as the treatment of choice [1].

ER signaling is complex

Originally, it was considered that there was only one ER isoform, now known to be ERα, and this was used to identify ER-positive tumors. However, the discovery of a second ER isoform, ERβ [2], has indicated that the ER signaling pathway is more complex than was initially envisaged. Structurally the two isoforms are highly homologous, even though human ERα is longer than ERβ (595 amino acids versus 530 amino acids, respectively) and there are distinct differences in the hormone-binding domain and the amino terminus (where the activation factor-1 region is located, Figure 1a). Following binding to 17β-estradiol, ERα and ERβ both mediate gene transcription via

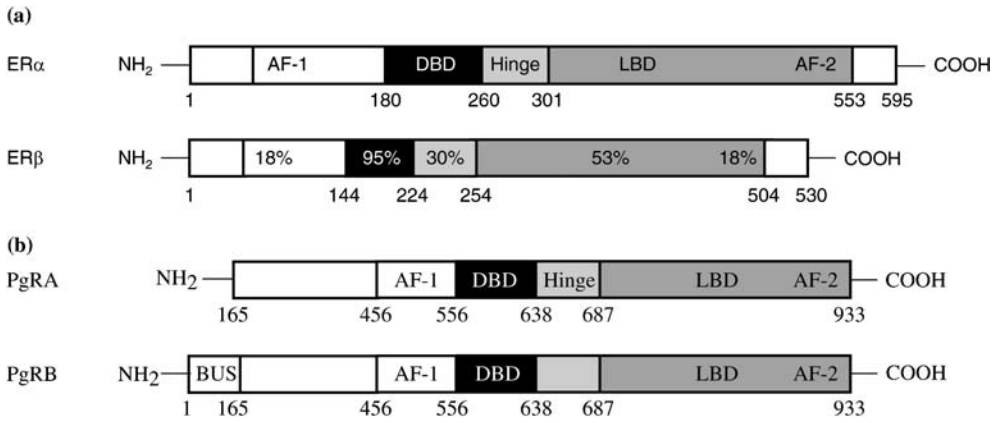

Figure 1. Comparative structures of ER (a) and PgR (b) isoforms, showing domain homology between the isoforms. Abbreviations: AF-1 and AF-2, activator function 1 and 2; DBD, DNA-binding domain; LBD, ligand-binding domain; BUS, B-upstream segment.

estrogen response elements (ERE); however, while ERα can also activate gene transcription from the AP-1 site, ERβ cannot [3]. As such, it is important to consider how ERα and ERβ interact and whether their differential expression has any clinical significance.

The expression and interaction of ERα and ERβ

Several studies have found predominant ERβ expression in normal breast tissue, while in contrast most breast tumors expressed ERα [4, 5]. These observations suggest that ERβ may act as a natural suppressor of ERα [6, 7]. ERα is expressed at an early stage in breast cancer development [8], and in primary tumors ERα expression is generally higher than ERβ, although the distribution of expression is similar between the two isoforms [9]. Indeed, several studies have found that the majority of tumors co-express both ERα and ERβ (Figure 2) [5, 9–12]. Interestingly, it has been observed that ERβ expression is lost in ~20% of metastatic breast cancer tumors that were initially ERβ-positive, particularly in ductal carcinoma [13]. Overall, these observations indicate that ERα and ERβ expression, distribution and interaction may modulate tumor development. It is therefore important to identify how the expression of ERα or ERβ might affect the clinical outcome and perhaps ultimately

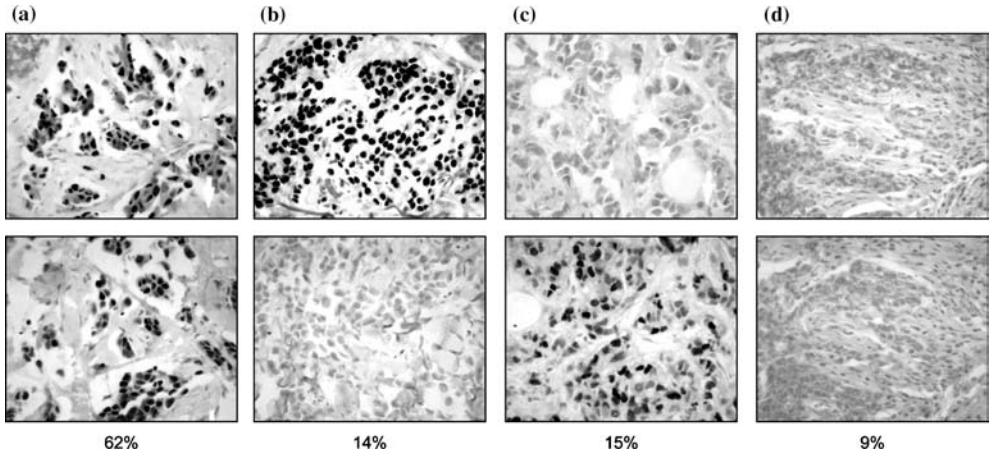

Figure 2. Differential expression of ERα (upper sections) and ERβ (lower sections) in breast cancer and the percentage of tumors expressing both ERα and ERβ (a), ERα only (b), ERβ only (c), and neither ERα nor ERβ (d) [9]. Reproduced with permission of Cancer Research.

to use this information to determine a patient's optimal treatment regimen.

ER isoforms can affect the clinical outcome of breast cancer

Initially, ERα represents a favorable prognostic factor and is a useful predictive factor for responses to endocrine therapies such as tamoxifen (Figure 3) [14]; however, after ~5 years the prognostic value of ERα expression lessens [15], indicating that with long-term follow-up the value of biomarkers such as ERα may change. The importance of ERα is further highlighted by observations that occasional loss of ERα expression is associated with the acquisition of endocrine resistance, although the mechanisms are poorly understood [7].

Whether ERβ expression is associated with response to endocrine therapy remains an area of debate, and while some studies have found that ERβ-positive tumors may be less responsive to tamoxifen [4], others have suggested that ERβ tumors are likely to respond to endocrine therapies [11]. The overall situation may be further complicated by ERβ-mediated suppression of ERα; how this affects clinical outcome remains an area of discussion, although some studies suggest that ERβ expression may be associated with a favorable prognosis [16, 17]. For example, ERβ reduced the expression of estrogen-induced, proliferation-related genes such as cathepsin D [16], whose overexpression has been associated with poor prognosis [18]. Additionally, some reports have indicated that survival is significantly improved in patients whose tumors express ERβ [17, 19], although further analyses using larger numbers of patients are required.

An analysis using large patient numbers has observed that ERβ may not correlate with the clinical features usually associated with ERα, such as diploidy and small tumor size, both of which are associated with a favorable prognosis [9]. Evidence from this study also implied that ERβ expression is not a surrogate marker for ERα, as significant relationships between ER isoforms and prognostic factors (such as tumor grade, proliferation [Ki67 labeling], S-Phase fraction or DNA ploidy) were only observed for ERα [9]. Indeed, the association of ERβ with aneuploidy indicates that ERβ-positive tumors may be more aggressive, in contrast with previous research suggesting a favorable prognosis with ERβ expression [16]. Further investigations that analyze ER isoform expression in particular patient subgroups may further clarify the relative clinical significance of ERα and ERβ.

ER variants and mutations provide additional complexities

ER isoform expression is further complicated by ERα and ERβ variants, for example ERδE7, ERβ2 (Erβcx) and ERβ3-5 [20, 21]. The identified ERα variant, ERδE7 [22], inhibits ERE-driven transcription, although how this may affect the clinical outcome of breast cancer has yet to be clarified [23]. There is a fairly large amount of preclinical

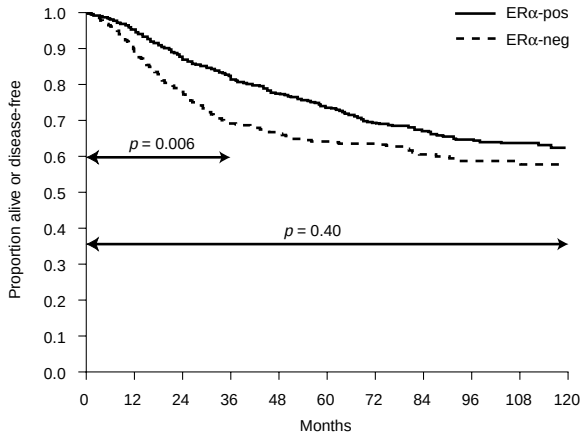

Figure 3. Kaplan–Meier curves of disease-free survival in ERα-positive patients treated with adjuvant anastrozole or tamoxifen [15]. Reproduced with kind permission of Kluwer Academic Publishers.

literature surrounding ERβ variants, although few studies have analyzed ERβ variants in clinical tumor samples. One study found increased ERβcx expression in tumor biopsies compared with normal breast tissue [24] and another study using Western blot analysis found evidence that ERβcx expression may possibly affect the response to tamoxifen [25]. However, another study did not find many tumor biopsies with variant ERβ expression (Prof Fuqua, unpublished data). Larger studies are needed in order to clarify the precise impact of ERβ variants in breast cancer.

The overall picture is further complicated by the expression of a mutated form of ERα that is hyperresponsive to estrogen, and also by the presence of co-activators and co-repressors of ER function. A common somatic ERα mutation (A908G) that occurs in early premalignant breast lesions (identified in 34% of hyperplasia biopsies) has increased sensitivity to estrogen when compared with wild-type ERα [26]. Thus, ERα A908G may promote or accelerate the progression of pre-malignant to malignant breast lesions. However, an analysis of over 300 biopsies (only 14 of which were hyperplasia biopsies) did not detect ERα A908G [27], although it is unknown whether the method used in this study was sufficient for detection of the mutant, compared to the genomic sequencing used in the earlier study. Further studies with comparable sample numbers for the different stages of breast cancer are required to clarify the impact of specific ER mutants.

Co-activators of ER function include AIB1 (which reduces the antagonist activity of tamoxifen) whose high expression in tamoxifen-treated patients has been associated with worsened survival, indicative of tamoxifen resistance [28]. In contrast, high AIB1 expression in patients who did not receive tamoxifen was associated with longer survival [28]. These data highlight the complex nature of ER-mediated signaling upon clinical outcome, and emphasize that multifactorial approaches may be required to identify factors associated with improved clinical outcome.

PgR signaling has an impact upon breast cancer

As with the ER, the PgR has also been shown to exist in two isoforms, PgRA and B [29]. Two distinct promoters (both under the control of estrogen) transcribe the two isoforms, which differ in that PgRA is smaller than PgRB, lacking 164 amino acids from the N-terminus (Figure 1b) [30, 31] and they have different transcriptional activities when expressed in cells.

How the expression and interaction of PgR isoforms may affect clinical outcome

Preliminary studies of the PgR isoforms have begun to demonstrate the value of the relative expression of PgRA and PgRB. In the neoplastic breast, the proportion of cells expressing PgRA and/or PgRB is reduced in ductal carcinoma *in situ* (65 and 75%, respectively) and invasive ductal carcinoma (66 and 55%, respectively) compared with proliferative disease without atypia (85 and 96%, respectively) and atypical ductal hyperplasia (100 and 100%, respectively) [32]. In invasive ductal carcinoma, PgRA and PgRB expression was associated with histological grade and the positive correlation between PgRA and ERα suggests that the two PgR isoforms may be differently regulated by estrogens [32]. Genes that are known to confer susceptibility to developing breast cancer also affect expression of PgR. For example, BRCA1 or BRCA2 mutation results in PgRA predominance, raising the possibility that changes in progesterone signaling may be involved in the increased risk of cancer observed in women with BRCA1 or BRCA2 mutations [33]. As such, levels of PgRA and PgRB may affect the clinical outcome of patients with breast cancer, as well as those who are more likely to develop breast cancer.

The PgRA : PgRB ratio has also been suggested to be of relevance in influencing the biological actions of progesterone, and a recent study has examined this in a group of patients with advanced breast cancer receiving tamoxifen [34]. When disease-free survival is examined in relation to the PgRA : PgRB ratio, patients with a ratio of ≤ 1 do significantly better than those with a ratio >1 ($p = 0.0209$) (Figure 4) [34]. These preliminary data demonstrate that the relative expression of different hormone receptor isoforms may have clinical value as a predictive biomarker.

Expression of PgR variants and mutations in relation to clinical outcome

Although several variants and mutations/polymorphisms of PgR isoforms have been identified

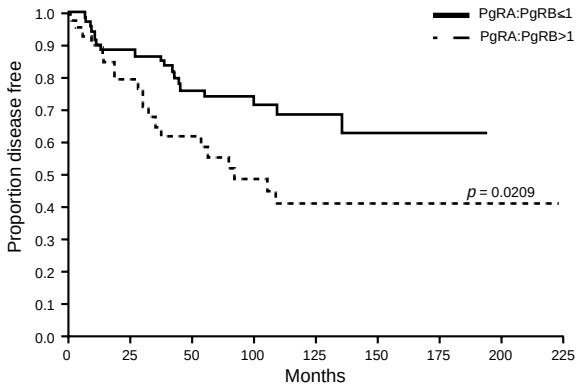

Figure 4. Kaplan–Meier curves of disease-free survival in tamoxifen-treated patients stratified by PgRA:PgRB ratio [34]. Reprinted with permission from Clin Cancer Res.

[35, 36], there are little published data regarding their effect upon clinical outcome. Some reports have observed increased tumor expression of variant PgR (PgRδ6,2) when compared with normal tissue from patients with breast cancer [36], although the effect of this variant PgR upon clinical outcome was not described. Contrasting effects of PgR polymorphisms upon the risk of developing breast cancer have been noted; some studies have found that PgR G660T protects against breast cancer [37], while others did not [38–40]. Whether the expression of PgR mutants affects clinical outcome has yet to be reported.

Overall, the complexity of the cellular environment in which ER and PgR receptors function cannot be underestimated. Where once it was considered that only one ER and PgR was involved in determining response, we now know that the situation is significantly more complex and this emphasizes the difficulty in translating such findings into the clinic.

Multifactorial approaches that identify clinical biomarkers

Ultimately, it can be argued that the use of single biomarkers will be limited, particularly when the amount of 'cross-talk' between different cell-signaling pathways, such as the epidermal growth factor family of receptors, is considered [41]. In addition, there are multiple receptor isoforms involved in regulating the response of tumors to hormonal stimuli as well as mutant and variant receptors. Therefore, multifactorial approaches may identify and characterize markers more precisely, so that different patient groups can be accurately identified and appropriately treated. The use of genomic- or proteomic-based approaches to produce molecular portraits of breast cancers indicates an alternative systematic approach to identifying important markers of response and prognosis. Instead of looking at the expression of one or two isolated genes, these approaches allow the examination of large numbers of genes or proteins in a single experiment.

Using DNA microarrays to predict the optimal treatment option

The application of cDNA microarrays to the problem of predicting the optimal treatment of choice for an individual with breast cancer has already shown a great deal of promise, with gene-expression profiles that predict for chemotherapy sensitivity already identified [42]. In a large analysis of $\sim$ 5000 genes from 98 patients, two distinct tumor groups were identified for which the likelihood of developing distant metastases within 5 years was lower in one group (34%) than the other (70%) [43]. When these two distinct tumor groups were associated with histopathological data, the majority of tumors expressing ERα were from patients with a low likelihood of developing distant metastases within 5 years. Thus gene expression can be a better predictor of survival than conventional approaches, based on both clinical and histological criteria [43, 44].

High-throughput proteomic studies can identify pre-malignant proteins

In comparison to genomic approaches, high-throughput proteomic studies are at a relatively early stage of development, although initial results have been promising. In a preliminary investigation comparing total proteins from four women with infiltrating ductal carcinoma with proteins from a woman with no history of breast cancer [45], 524 proteins were identified that showed a ≥3-fold abundance in infiltrating ductal carcinoma compared with normal tissue, along with a number of proteins that were commonly expressed by malignant tissues. Thus, quantitative and qualitative differences in protein abundance between infiltrating ductal carcinoma and normal tissue have been shown. Further proteomic studies with larger patient numbers that relate proteomic analysis to clinical outcome will be of great interest.

Conclusions

We know that ER and PgR expression affect the clinical outcome of breast cancer. However, ER and PgR are not the only proteins involved in the development and metastasis of breast cancer, as approximately 30–40% of ER and/or PgR-positive tumors do not respond to endocrine therapy [2]. This may be explained by the expression of different isoforms of ER and PgR, or cross-talk between other signaling pathways such as the epidermal growth factor receptor pathway [41]. Therefore, to optimize clinical outcome, multifactorial approaches are needed in order to characterize biomarker expression in tumor samples. Recent developments in genomic and proteomic methods offer the chance for this to occur. The identification of different genetic signatures that predict clinical outcome may quickly delineate the signaling pathways involved in disease progression, enabling patterns of biomarkers to be identified, along with markers of diagnosis and response [46]. While these approaches are excellent hypothesis-generating studies, it is also crucially important to determine their significance on a wider, clinical scale. In this way, the information gained from such studies may shape clinical practice in the future and provide an optimal treatment plan for each individual with breast cancer.

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