Is oestrogen receptor-β a predictor of endocrine therapy responsiveness in human breast cancer?

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Abstract

The role of oestrogen receptor (ER)β in human breast cancer remains unclear. However, it is now apparent that when considering ERβ in human breast cancer it is important to recognise two ERβ expressing groups, one in which ERβ is co-expressed with ERα and the other where ERβ is expressed alone. Emerging data support different functions between ERβ when it is expressed alone and when it is co-expressed with ERα. With regard to the latter group (ERα+/ERβ+), there are now 9 out of 10 retrospective clinical outcome studies published, that support the hypothesis that increased expression of ERβ is associated with increased likelihood of response to endocrine therapy. The data strongly support undertaking prospective studies to determine if the addition of ERβ to ERα is clinically beneficial and whether to include both ERβ and ERα when establishing clinically relevant cut-offs for defining ER status.

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Introduction

Oestrogens are pivotal in the growth and development of both normal and neoplastic mammary tissues (Kelsey 1993), and mediate most of their action via ligand-dependent transcription factors called oestrogen receptors (ER). In pathological lesions associated with increased risk of breast cancer, there is a significant increase in the proportion of cells that express ER (Khan et al. 2002, Shaaban et al. 2002). Also ER expression is present in approximately 70% of invasive breast cancers. Importantly, ER is the primary target for both chemoprevention and endocrine therapy of breast cancer (Ali & Coombes 2002). The ER status of breast tumours (determined as a binary factor of − or +) provides prognostic information but more importantly is a predictor of response to endocrine therapy (Ali & Coombes 2002). The former role may relate to an indirect association between ER expression and a distinct cell ‘circuitry’ associated with a ‘well differentiated’ morphological appearance. The second relates to the central role of ER within the oestrogen signaling pathway so that ER inactivation inhibits oestrogen signaling and the proliferative action of oestrogen on ER+ human breast cancer cells (Lippman & Bolan 1975). This is the rationale for measuring ER and progesterone receptor (PR), a down-stream marker of functional ER signalling (Horwitz et al. 1978), routinely in breast cancer biopsies. In addition, ER− status is associated with specific subsets of in situ and invasive breast cancer that display aggressive biological behaviour (Holland et al. 1997, Lapidus et al. 1998). Therefore, despite the fact that ER status is already an important biomarker in breast cancer, (Ali & Coombes 2002), it is now appreciated to be far more complex. There are now two known ERs – ERα and ERβ (Ali & Coombes 2002) which has led to a re-evaluation of oestrogen action in target tissues such as breast tumours.

With respect to human breast cancer, the previous and current assays used for determining ER status in most cases generally detect only ERα (see ER assays below) but the newer ERβ is clearly expressed in both normal and neoplastic human breast tissue (Leygue et al. 1998, Jarvinen et al. 2000), although...
its role in either is unknown. In animal studies, while ERα has been shown to be essential for normal mammary gland development, ERβ effects are more subtle, with roles in terminal differentiation (Forster et al. 2002) and modulation of ERα activity being described (Hall & McDonnell 1999, Weihua et al. 2000, Peng et al. 2003). However, ERβ effects on mammary tumourigenesis in animal models have not been reported. In this review, we discuss data concerning ERβ expression in human breast cancer in vivo and its relationship to clinical outcome in order to gain insight into the putative function(s) of ERβ in human breast cancer. A review has recently been published which also deals with the clinical significance of ERβ and its isoforms in breast cancer (Saji et al. 2005). The current review builds on this by incorporating additional studies and by focusing specifically on ERβ, the relationship between clinical ER assays and endocrine therapy outcome, and the likelihood that ERβ in addition to ERα may be a clinically useful biomarker of treatment responsiveness in breast cancer.

**ER assays**

Traditionally, whole tissue extract/cytosol-based radioligand-binding assays (LBA, dextran-coated charcoal assay, DCC) were used to determine ER and PR in biopsies. In these assays ER is measured in fmol/mg protein and cut-offs of between 3–10 fmol/mg protein (based on clinical response data) (Osborne 1998) are used to define ER status as negative or positive. But, absolute levels of ER also provide relevant predictive information, increasing ER levels are associated with the increased likelihood of response to endocrine therapies (Osborne 1998). Since ERα and ERβ bind radiolabelled oestradiol-17β similarly, such assays do not discriminate between ERα and ERβ. In most cases, the level of ERα RNA greatly exceeds (10–100 times) ERβ RNA in breast tumours (Ariazi et al. 2002) leading to the assumption that ERα protein levels greatly exceed ERβ protein levels. In general, ERα is up-regulated and ERβ is down-regulated in breast tumours, it is not surprising that LBAs correlate well with ERα RNA levels (Dotzlaw et al. 1990). It is generally thought that LBAs most often detect ERα with little interference from ERβ (Brouillet et al. 2001). However, exceptions may occur (Saji et al. 2002a,b). Newer methods using specific monoclonal antibodies raised against ERα allow detection of ER, both in whole tissue/cytosolic extracts (ELISA) and in tissue sections using immunohistochemistry (IHC). These antibody-based assays generally correlate well with LBA in breast tumour extracts (Osborne 1998). The biochemical assays (DCC and EIA) as well as IHC methods, while having specific advantages and disadvantages, produce information that is useful for therapeutic decision making. DCC assays measure ER level and function (ligand-binding ability), but relatively large amounts of biopsy tissue are required, a problem with respect to the overall trend to the decreased size of breast tumours over the last few years due to earlier detection technologies. Also, this assay does not account for tumour heterogeneity and therefore contribution from different elements other than invasive tumour cells, such as normal and/or preneoplastic breast cells, in addition to vascular and immune cells are unknown. In contrast, IHC uses 5 μm sections of a biopsy, can localize positive cells and their relative proportion in the tumour. However, quantification is imprecise and the information derived is fundamentally different from the DCC assay. In fact, we have previously shown that ERα status measured by IHC can be different with different antibodies and that the discrepancies can be correlated in some cases with the expression of ERα variant mRNA that might encode proteins recognised by only a subset of ERα antibodies (Huang et al. 1997, 1999). Nevertheless, with good correlation between the assays established, and sensitive ERα antibodies available, ERα is generally measured by current assays and ERα is correlated with prognosis and treatment response (Harvey et al. 1999, Osborne 1998) in breast tumours. However, with increasing evidence of a role of ERβ in breast cancer (see below), we would argue that ER status should now include both ERα and ERβ.

**Assessment of ERβ expression**

In contrast to ERα, published data suggest that ERβ expression declines during breast tumourigenesis (Leygue et al. 1998, Roger et al. 2001). This general downregulation of ERβ in breast tumours compared with normal breast tissue, suggests a role for ERβ as a tumour suppressor (Skliris et al. 2003). Nevertheless, ERβ expression in breast tumours varies widely (Dotzlaw et al. 1999, Jarvinen et al. 2000) and attempts to correlate ERβ with various biomarkers has resulted in varied, often contradictory conclusions (Speirs 2002). In
part, this is due to how ERβ expression was determined (RNA or protein) and/or which antibody was used (Skliris et al. 2002). The latter is important since variant non-ligand binding ERβ proteins have been detected in breast tissues (Fuqua et al. 1999, Saji et al. 2002b) and their function may be distinct from that of the full-length ligand-binding ERβ1 protein (Fig. 1) (Saji et al. 2002a, Peng et al. 2003). Therefore depending on the antibody used, full-length ERβ only or total (full-length plus variants) ERβ is determined. Also, assay protocols and criteria for scoring ERβ status (+ or −) varies among studies (Jarvinen et al. 2000, Mann et al. 2001, Fuqua et al. 2003, Carder et al. 2005), and there are no prospectively obtained clinically relevant cut-off values.

Several ERβ specific antibodies have now been used for IHC to detect ERβ-like proteins. Skliris et al. (2002) have previously compared various antibodies for their usefulness in IHC. They found that for formalin-fixed paraffin embedded tissues Ab288/14C8 (Fuqua et al. 1999) and MCA1974S/PPG5/10 (Saunders et al. 2002; see Fig. 1 for regions of ERβ used to generate antibodies) both commercially available from Abcam and Serotec, respectively, gave the most superior nuclear staining. Both these antibodies have been validated for their specificity (Saunders et al. 2002, Skliris et al. 2002, G Weitsman, G Skliris, K Ung, B Peng, M Younes, P H Watson & L C Murphy, unpublished observations). However, other antibodies were found more reliable for frozen tissue IHC or for Western blotting, immunoprecipitation or FACS analysis (Skliris et al. 2002, G Weitsman, G Skliris, K Ung, B Peng, M Younes, P H Watson & L C Murphy, unpublished observations). This previous study did not include an evaluation of the rabbit polyclonal GC17/385P ERβ antibody (Biogenex, USA; see Fig. 1), which has also been rigorously validated for its specificity and usefulness in formalin-fixed paraffin-embedded tissue sections (Leav et al. 2001). We routinely use 14C8 and GC17 ERβ antibodies for the specific immunohistochemical evaluation of ERβ in human breast tumour sections and tissue microarrays using tumours processed by and stored in the Manitoba Breast Tumour Bank (Watson et al. 1996, Murphy et al. 2002, Skliris et al. 2005, G Weitsman, G Skliris, K Ung, B Peng, M Younes, P H Watson & L C Murphy, unpublished observations). However the equally important issues are the differences in protocols and procedures that are used for immunohistochemical analysis of ERβ as underscored by Speirs et al. (Carder et al. 2005).

**Figure 1** Schematic representation of human ERα and β isoform proteins. Human ERβ1 is in the full-length ligand binding form, and hERβ2/cx and hERβ5 are two commonly occurring C-terminally truncated non-ligand binding variants. 14C8 was made to peptide consisting of amino acids 1–153 of long form of ERβ; GC17 to amino acids 449–465; PPG5 to amino acids 512–530.
Table 1 Summary of retrospective studies of ER\(\beta\) protein expression and clinical outcome in breast cancer

<table>
<thead>
<tr>
<th>Author/Reference</th>
<th>Number of patients in study</th>
<th>ER(\beta) detection (antibody)</th>
<th>Treatment</th>
<th>Clinical outcome ((P) value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann et al. (2001)</td>
<td>118</td>
<td>IHC (N)</td>
<td>Tam</td>
<td>ER(\beta)+ associated with increased OS (0.0077)</td>
</tr>
<tr>
<td>Murphy et al. (2002)</td>
<td>27</td>
<td>IHC (N)</td>
<td>Tam</td>
<td>Higher ER(\beta) associated with no disease progression (0.046)</td>
</tr>
<tr>
<td>Omoto et al. (2001)</td>
<td>88</td>
<td>IHC (C)</td>
<td>Tam &amp; Chemo</td>
<td>ER(\beta)+ associated with increased DFS (0.032)</td>
</tr>
<tr>
<td>Fleming et al. (2004)</td>
<td>52</td>
<td>IHC (C)</td>
<td>Tam</td>
<td>ER(\beta)+ associated with no relapse within 5 years (&lt;0.05)</td>
</tr>
<tr>
<td>Esslimani-Sahla et al. (2004)</td>
<td>50</td>
<td>IHC (N)</td>
<td>Tam</td>
<td>Higher ER(\beta) associated with no relapse within 5 years (0.004)</td>
</tr>
<tr>
<td>Hopp et al. (2004)</td>
<td>186</td>
<td>IHC (N)</td>
<td>Tam</td>
<td>Higher ER(\beta) associated with improved DFS (0.0063) and OS (0.03)</td>
</tr>
<tr>
<td>Iwase et al. (2003)</td>
<td>77</td>
<td>IHC (C)</td>
<td>Tam or other endocrine therapies</td>
<td>ER(\beta)+ show better response to endocrine therapy (0.088)</td>
</tr>
<tr>
<td>O’Neill et al. (2004)</td>
<td>138</td>
<td>IHC (C)</td>
<td>Tam</td>
<td>ER(\beta)+ show worse OS (0.09)</td>
</tr>
<tr>
<td>Myers et al. (2004)</td>
<td>150</td>
<td>IHC (C)</td>
<td>Tam &amp; Chemo</td>
<td>ER(\beta)+ associated with increased DFS (0.0008)</td>
</tr>
<tr>
<td>Nakopoulou et al. (2004)</td>
<td>181</td>
<td>IHC (C)</td>
<td>Tam &amp; Chemo</td>
<td>ER(\beta)+ associated with increased DFS (0.0002) and OS (0.0002)</td>
</tr>
</tbody>
</table>

N, antibody detects an N-terminal ER\(\beta\) epitope; C, antibody detects a specific C-terminal epitope of the full-length ligand binding ER\(\beta\)1 protein; Tam, tamoxifen; chemo, chemotherapy; IHC, immunohistochemistry; WB, western blot. DFS, disease free survival; OS, overall survival.

ER\(\beta\) expression and its potential role as a predictor of treatment response in breast cancer

The marked discrepancy between conclusions drawn from studies of ER\(\beta\) measured by RNA compared with those where ER\(\beta\) was measured at the protein level has been underscored by Saji et al. (2005). As noted by Saji et al. (2005) no clear explanation exists. However such discrepancies may be due to the fact that when RNA is measured in extracts from either breast tissue sections or chunks of tissue, the RNA represents a pool of all the different cell types present in the biopsy e.g. stromal vascular cells, infiltrating lymphocytes, most of which will also express ER\(\beta\), and normal, preinvasive and invasive epithelial cells, where the extent of contaminating normal epithelium may have a significant influence,(Roger et al. 2001, Murphy et al. 2002, Skliris et al. 2002). It is also possible that some factors involved in changing the sensitivity of IHC to some antigens are not relevant to ER\(\beta\) RNA assays (McCabe et al. 2005). However, most determinants of ER\(\beta\) protein expression in breast tumours are done using IHC which allows expression in invasive breast cancer cells to be assessed independently of other cell types. Therefore for the purposes of the following discussion only those studies, where ER\(\beta\) protein was determined (IHC or in one study by western blotting), are considered.

A common, but not universal finding, is that ER\(\beta\) expression correlates with ER\(\alpha\) and PR expression (Jarvinen et al. 2000, Murphy et al. 2002, Omoto et al. 2002), which are good prognostic and treatment response biomarkers. While the role of ER\(\beta\) in breast cancer is unclear, one important currently emerging hypothesis is that increased expression of ER\(\beta\) is associated with increased likelihood of response to endocrine therapy.

As of January 2006 there are ten retrospective studies that have assessed ER\(\beta\) expression in relation to clinical outcome associated with endocrine therapy in breast cancer (Table 1). Seven of these assessed ER\(\beta\) expression in relation to responsiveness to tamoxifen therapy or other endocrine therapies (Mann et al. 2001, Murphy et al. 2002, Iwase et al. 2003, Esslimani-Sahla et al. 2004, Fleming et al. 2004, Hopp et al. 2004, O’Neill et al. 2004). Three assessed ER\(\beta\) expression with respect to disease free survival in patients who were treated with chemotherapy and tamoxifen (Omoto et al. 2001, Myers et al. 2004, Nakopoulou et al. 2004). No formal meta-analysis has been done, but in the first group of studies, five out of seven found that increased levels of ER\(\beta\) were associated with a better disease outcome and consistent with the breast tumour being tamoxifen sensitive (Mann
et al. 2001, Murphy et al. 2002, Esslimani-Sahla et al. 2004, Fleming et al. 2004, Hopp et al. 2004). The patient/tumour characteristics are of course mixed but the total combined patient number in these five ‘positive’ studies is 433, compared with 138 patients in the one ‘negative’ study, where no significant difference was found although there was a trend to worse outcome in those patients whose tumours had higher ERβ expression (P = 0.09; O’Neill et al. 2004) and 77 patients in the other ‘not significant’ study, where a trend toward higher ERβ was being associated with better response to tamoxifen (P = 0.088; Iwase et al. 2003) was found. There are also three studies (Omoto et al. 2001, Myers et al. 2004, Nakopoulou et al. 2004) in which ERβ expression in primary tumours was compared with disease free survival but where the patient’s treatment included chemotherapy plus tamoxifen. All these studies show a significant association of higher ERβ expression and better disease free survival, with total accumulated patient numbers of 419. Three of the above studies (Mann et al. 2001, Hopp et al. 2004, Nakopoulou et al. 2004) also show a significant association of higher ERβ expression and overall survival. So there are nine studies published where the conclusion was made that ERβ expression in breast tumours is associated with better disease outcome of the patients (n = 929) compared with one study where ERβ expression is associated with worse disease outcome (n = 138).

However, when considering ERβ expression in breast cancers in vivo there are two groups, one where ERβ is coexpressed with ERα (ERβ+/ ERα+) and the other where ERβ is expressed alone (ERβ+/ ERα−). The first group, (ERβ+/ ERα+), comprises ~59% of primary human breast cancers (Murphy et al. 2003, Saji et al. 2005), while the ERβ alone expressing group comprises ~17% of breast cancers (Murphy et al. 2003, Saji et al. 2005). Generally it is only patients whose tumours are ER+ that are treated with tamoxifen (or other endocrine therapies) and ER+ status is determined only by ERα (specific ERα antibodies are used in current IHC clinical assays, as discussed above). Therefore the majority of tumours being assessed in the above studies are those co-expressing ERβ with ERα. These data support the hypothesis that assessment of ERβ together with ERα is a better predictor of endocrine responsiveness than ERα alone. In addition, since some studies suggest that ERβ correlates with and also regulates PR expression together with ERα (Jarvinen et al. 2000, Omoto et al. 2001, Murphy et al. 2002, 2005) it may be that ERβ and ERα are better biomarkers than ERα and PR, or alternatively the three biomarkers in combination may be the most precise predictors of endocrine responsiveness. However since the clinical studies discussed above were small, retrospective, used different protocols, antibodies and cut-off values to determine positive or negative ERβ status, a definitive conclusion regarding the addition of ERβ to ERα as a better predictor of endocrine response than ERα alone cannot be made and there is insufficient evidence to enable incorporation of a promising ERβ assay into the clinic.

The second group of ERβ expressing tumours (ERβ+/ ERα−) would traditionally be classified as ER−. ER− tumours are more aggressive and less than 10% respond to endocrine therapies (Osborne 1998). However, now it appears that greater than 50% of all tumours previously classified as ER− express ERβ (Murphy et al. 2003). It has been suggested that some of the so called ER− tumours where the patient responded to endocrine therapy were due to false negative assays for technical reasons (Muschenheim et al. 1978, Clarke et al. 2001), or ER-independent mechanisms associated with tamoxifen action (Clarke et al. 2001), however, a role of ERβ in this group of tumours is now another possibility. ERβ+/ ERα− tumours generally have received very little attention, however, there are three studies (Jensen et al. 2001, O’Neill et al. 2004) including our own unpublished study (Skliris et al. 2005) with a total combined 389 cases where ERβ+ alone expressing tumours have been investigated. All three studies identified a significant association of ERβ expression with Ki67, a marker of proliferation, and one study also identified a significant association of ERβ with cyclin A expression (Jensen et al. 2001). Such data suggest that the role of ERβ in the absence of ERα expression in breast cancer is different to that when ERβ is co-expressed with ERα. Furthermore a different function of ERβ when expressed alone is also supported mechanistically, since when ERβ is co-expressed with ERα they will form heterodimers (Cowley et al. 1997) preferentially. Recent fluorescence resonance energy transfer (FRET) analyses showed formation of both ERα and ERβ homo- and hetero-dimers in situ in living cells in culture (Bai & Giguere 2003). This activity may underlie observations that ERβ often but not always, has a negative modulatory effect on ERα when co-expressed (Ogawa et al. 1998, Hall & McDonnell 1999, Peng et al. 2003). In contrast, when ERβ is expressed alone it will homodimerize. Distinct as
well as overlapping activity, as assessed by determining gene expression profiles using DNA microarray analyses, has been demonstrated for each type of ER homodimer and ERβ/ERα heterodimers in osteosarcoma cell line models, engineered to express ERβ alone, ERα alone, or both ERβ and ERα (Monroe et al. 2005). An implication of these data is the possibility that targeting ERβ pathway(s) in the ERα− but ERβ+ group of patients may be a treatment option for these patients who generally have few options other than aggressive chemotherapies. However, further studies are required with respect to this issue.

Conclusion

When considering ERβ in human breast cancer it is important to recognise two ERβ expression cohorts since there are emerging data supporting differential function of ERβ when it is expressed alone compared with when it is co-expressed with ERα. While the differential role of ERβ in the absence of ERα is intriguing and deserves continued study, the retrospectively collected data summarised above investigating the relationship between ERβ when coexpressed with ERα and clinical outcome, strongly support the undertaking of definitive prospective studies to determine if the addition of ERβ to ERα is clinically beneficial, and if so to establish clinically relevant cut-off values for defining ER status to include both ERβ and ERα. Such studies require standardized approaches (Carder et al. 2005) reagents, protocols and cutpoints (McCabe et al. 2005) and equipment routinely available to a clinical pathology laboratory to enable incorporation of a promising ERβ assay into the clinic.

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