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 ER β in human breast cancer it is important to recognise two ER β expressing groups, one in which ER β is co-expressed with ER α and the other where ER β is expressed alone. Emerging data support different functions between ER β when it is expressed alone and when it is co-expressed with ER α . With regard to the latter group (ER α +/ER β +), there are now 9 out of 10 retrospective clinical outcome studies published, that support the hypothesis that increased expression of ER β is associated with increased likelihood of response to endocrine therapy. The data strongly support undertaking prospective studies to determine if the addition of ER β to ER α is clinically beneficial and whether to include both ER β and ER α 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 – ER α and ER β (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 ER α (see ER assays below) but the newer ER β 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 ER α has been shown to be essential for normal mammary gland development, ER β effects are more subtle, with roles in terminal differentiation (Forster *et al.* 2002) and modulation of ER α activity being described (Hall & McDonnell 1999, Weihua *et al.* 2000, Peng *et al.* 2003). However, ER β effects on mammary tumourigenesis in animal models have not been reported. In this review, we discuss data concerning ER β expression in human breast cancer *in vivo* and its relationship to clinical outcome in order to gain insight into the putative function(s) of ER β in human breast cancer. A review has recently been published which also deals with the clinical significance of ER β 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 ER β , the relationship between clinical ER assays and endocrine therapy outcome, and the likelihood that ER β in addition to ER α 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 ER α and ER β bind radiolabelled oestradiol-17 β similarly, such assays do not discriminate between ER α and ER β . In most cases, the level of ER α RNA greatly exceeds (10–100 times) ER β RNA in breast tumours (Ariazi *et al.* 2002) leading to the assumption that ER α protein levels greatly exceed ER β protein levels. In general, ER α is up-regulated and ER β is down-regulated in breast tumours, it is not surprising that LBAs correlate well with ER α RNA levels (Dotzlaw *et al.* 1990). It is generally thought that LBAs most often detect ER α with little interference from ER β (Brouillet *et al.* 2001). However, exceptions may occur (Saji *et al.* 2002a,b). Newer methods using specific monoclonal antibodies raised against ER α allow detection of

ER, both in whole tissue/cytosolic extracts (ELISA) and in tissue sections using immuno-histochemistry (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 μ m 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 ER α status measured by IHC can be different with different antibodies and that the discrepancies can be correlated in some cases with the expression of ER α variant mRNA that might encode proteins recognised by only a subset of ER α antibodies (Huang *et al.* 1997, 1999). Nevertheless, with good correlation between the assays established, and sensitive ER α antibodies available, ER α is generally measured by current assays and ER α is correlated with prognosis and treatment response (Harvey *et al.* 1999, Osborne 1998) in breast tumours. However, with increasing evidence of a role of ER β in breast cancer (see below), we would argue that ER status should now include both ER α and ER β .

Assessment of ER- β expression

In contrast to ER α , published data suggest that ER β expression declines during breast tumourigenesis (Leygue *et al.* 1998, Roger *et al.* 2001). This general downregulation of ER β in breast tumours compared with normal breast tissue, suggests a role for ER β as a tumour suppressor (Skiris *et al.* 2003). Nevertheless, ER β expression in breast tumours varies widely (Dotzlaw *et al.* 1999, Jarvinen *et al.* 2000) and attempts to correlate ER β with various biomarkers has resulted in varied, often contradictory conclusions (Speirs 2002). In

Table 1 Summary of retrospective studies of ER β protein expression and clinical outcome in breast cancer

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

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

ER- β expression and its potential role as a predictor of treatment response in breast cancer

The marked discrepancy between conclusions drawn from studies of ER β measured by RNA compared with those where ER β 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 β , 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, Skiris *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 β RNA assays (McCabe *et al.* 2005). However, most determinants of ER β 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 β protein was determined

(IHC or in one study by western blotting), are considered.

A common, but not universal finding, is that ER β expression correlates with ER α 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 β in breast cancer is unclear, one important currently emerging hypothesis is that increased expression of ER β is associated with increased likelihood of response to endocrine therapy.

As of January 2006 there are ten retrospective studies that have assessed ER β expression in relation to clinical outcome associated with endocrine therapy in breast cancer (Table 1). Seven of these assessed ER β 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 β 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 β 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 ER β expression ($P = 0.09$; O'Neill *et al.* 2004) and 77 patients in the other 'not significant' study, where a trend toward higher ER β 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 ER β 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 ER β 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 ER β expression and overall survival. So there are nine studies published where the conclusion was made that ER β expression in breast tumours is associated with better disease outcome of the patients ($n = 929$) compared with one study where ER β expression is associated with worse disease outcome ($n = 138$).

However, when considering ER β expression in breast cancers *in vivo* there are two groups, one where ER β is coexpressed with ER α (ER β +/ER α +) and the other where ER β is expressed alone (ER β +/ER α -). The first group, (ER β +/ER α +), comprises ~59% of primary human breast cancers (Murphy *et al.* 2003, Saji *et al.* 2005), while the ER β 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 ER α (specific ER α 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 ER β with ER α . These data support the hypothesis that assessment of ER β together with ER α is a better predictor of endocrine responsiveness than ER α alone. In addition, since some studies suggest that ER β correlates with and also regulates PR expression together with ER α (Jarvinen *et al.* 2000, Omoto *et al.* 2001, Murphy *et al.* 2002, 2005) it

may be that ER β and ER α are better biomarkers than ER α 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 ER β status, a definitive conclusion regarding the addition of ER β to ER α as a better predictor of endocrine response than ER α alone cannot be made and there is insufficient evidence to enable incorporation of a promising ER β assay into the clinic.

The second group of ER β expressing tumours (ER β +/ER α -) 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 ER β (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 ER β in this group of tumours is now another possibility. ER β +/ER α - 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 (Skiris *et al.* 2005) with a total combined 389 cases where ER β + alone expressing tumours have been investigated. All three studies identified a significant association of ER β expression with Ki67, a marker of proliferation, and one study also identified a significant association of ER β with cyclin A expression (Jensen *et al.* 2001). Such data suggest that the role of ER β in the absence of ER α expression in breast cancer is different to that when ER β is co-expressed with ER α . Furthermore a different function of ER β when expressed alone is also supported mechanistically, since when ER β is co-expressed with ER α they will form heterodimers (Cowley *et al.* 1997) preferentially. Recent fluorescence resonance energy transfer (FRET) analyses showed formation of both ER α and ER β homo- and hetero-dimers *in situ* in living cells in culture (Bai & Giguere 2003). This activity may underlie observations that ER β often but not always, has a negative modulatory effect on ER α when co-expressed (Ogawa *et al.* 1998, Hall & McDonnell 1999, Peng *et al.* 2003). In contrast, when ER β 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 ER β /ER α heterodimers in osteosarcoma cell line models, engineered to express ER β alone, ER α alone, or both ER β and ER α (Monroe *et al.* 2005). An implication of these data is the possibility that targeting ER β pathway(s) in the ER α - but ER β + 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 ER β in human breast cancer it is important to recognise two ER β expression cohorts since there are emerging data supporting differential function of ER β when it is expressed alone compared with when it is co-expressed with ER α . While the differential role of ER β in the absence of ER α is intriguing and deserves continued study, the retrospectively collected data summarised above investigating the relationship between ER β when coexpressed with ER α and clinical outcome, strongly support the undertaking of definitive prospective studies to determine if the addition of ER β to ER α is clinically beneficial, and if so to establish clinically relevant cut-off values for defining ER status to include both ER β and ER α . 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 ER β assay into the clinic.

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