

# Reply: Expression of oestrogen receptor beta proteins in human breast cancer biopsies

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## Sir

We welcome the comments of Speirs and her co-workers concerning the variation and expression of ER $\beta$  protein in breast cancer tissues as reported by ourselves (Saunders *et al*, 2002a) and Skliris *et al* (2001). Clearly, the differences may be caused by variations in sensitivity or specificity of the assays, or both, and it will be important to pinpoint the reason. If sensitivity (and the methodology which might lead to this is discussed below), then discrepancies may be resolved by determining appropriate cut-off values. On the other hand, differences in specificity may produce variations that are not simply related to ER $\beta$  expression. It may therefore be useful to add the following perspective.

In our laboratory we have used a polyclonal antibody raised to hinge domain of ER $\beta$  for several studies on human tissues (Critchley *et al*, 2001; Saunders *et al*, 2000). When this antibody was tested on breast tissue sections we detected expression of nuclear receptor in most sections within both normal and cancerous cells (unpublished observations). In 1998 two papers were published showing that several isoforms of ER $\beta$  can be encoded by mRNAs formed by alternative splicing at the 3' end of the gene (Moore *et al*, 1998; Ogawa *et al*, 1998). Furthermore, Ogawa *et al* (1998) showed that the protein of one of these forms (ER $\beta$ cx/ER $\beta$ 2), that lacks the ability to bind oestradiol, could be detected in tissue culture cells following transfection and could blunt the response to oestrogen if co-expressed with ER $\alpha$ . Because the peptide used to raise our polyclonal antibody could recognise both the wildtype ER $\beta$  and this variant isoform we repeated our studies on breast tissue using a monoclonal antibody raised against the C terminus of full-length ER $\beta$ 1 (Saunders *et al*, 2002a) which we have shown does not cross react with ER $\beta$ cx/ER $\beta$ 2 (Saunders *et al*, 2002b). Although

the biological significance of the expression of ER $\beta$  variant proteins is not known it is notable that expression of ER $\beta$ cx has been reported to occur in prostatic cancers (Fujimura *et al*, 2001) and we have detected expression in breast cancer biopsies (Saunders, Miller manuscript in preparation). Immunohistochemical evaluations based on antibodies the specificity of which is not well defined may therefore lead to mis-interpretation of the likelihood that a tissue will respond to oestrogens via full-length ER $\beta$ .

In our experience there are two other factors that can have an adverse affect on the quality and reliability of detection of ER $\beta$  protein namely tissue fixation and the specificity of secondary antibodies. Based on our experiences we believe poor tissue preservation (e.g. underfixation seen in the centre of large tissue fragments) and even over fixation (cross-linking of epitopes) can account for some of the variation in the detection of ER $\beta$  reported over the last few years. Western analyses can also be problematic. We have found that ER $\beta$  breaks down readily in solution and cannot withstand more than one freeze–thaw cycle, furthermore several secondary antibodies we have tried have given false positive bands, some with molecular weights close to ER $\beta$ , when tested on membranes without the addition of primary antibody.

Taking all these factors into account it is perhaps unsurprising that we are some way from deciding how the detection of ER $\beta$  should influence decisions regarding the oestrogenic responsiveness of tissues including the breast (and it is worth noting that the application of ER $\alpha$  required years of refinement). However, we are currently assessing the predictive value of our assay for ER $\beta$  in tumours from patients with breast cancer receiving neo-adjuvant treatment with tamoxifen.

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## Corrigendum

# Heparanase is a prognostic indicator for postoperative survival in pancreatic carcinoma

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