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Comments and Critique

Abnormal Oestrogen Receptor in Clinical Breast Cancer

THE OESTROGEN receptor (ER) is an excellent marker of differentiation. It predicts improved disease-free survival in breast cancer and, most importantly, predicts the likelihood of benefit from tamoxifen therapy. But there are still many key issues regarding ER to be considered. First, why are some breast tumours ER-negative? And second, why do some ER-positive tumours behave as if they are ER-negative (e.g. fail antioestrogen therapy), and some ER-negative tumours behave as if they are ER-positive [e.g. synthesise progesterone receptors (PR)]?

With respect to the apparent loss of ER, there are a number of possibilities that need to be examined. We could have a deletion in the DNA of the gene itself. We could have mutations or rearrangements of the gene. We could have methylation within the coding domain or the promoter region. We could have downregulation of transcription of ER mRNA at the promoter level, or an altered message such as that which occurs with alternative splicing. We must also consider aberrant function, in other words, outlaw receptors, perhaps inappropriately driving or blocking control of tumour cells.

ER DNA STUDIES

Koh *et al.* looked at 34 breast cancer patients by Southern hybridisation analysis and did not find any evidence for ER amplification or rearrangement [1], while Nembrot *et al.* reported evidence for a 1.6–3-fold amplification in 6 of 14 cases [2]. Falette *et al.* looked at methylation of the ER gene by Southern analysis and found different methylation patterns in normal breast and adjacent tumour tissue, and in ER-positive and ER-negative tumours, but there was no difference in receptor expression as a function of methylation [3]. Castagnoli *et al.* in 1987 found a *Pvu*II restriction fragment length polymorphism (RFLP) in the ER gene of 14 of 20 males [4]. Hill studied this same RFLP and found that it correlated with ER expression in 188 breast cancer patients [5]. However, Parl *et al.* found the *Pvu*II RFLP to be correlated with age but not ER expression in a smaller number of breast cancer patients [6]. In a follow-up study, Parl's group located the *Pvu*II RFLP within intron 1; this time no correlation with either age or ER expression was seen in 260 breast cancer patients [7]. Finally, Wanless *et al.* described a *Hind*II RFLP in the ER gene in a small percentage of breast cancer patients, which correlated with PR expression [8].

ER mRNA STUDIES

Bartlett-Lee *et al.* found a good correlation between ER mRNA, protein and ligand binding [9]. Rio *et al.*, by northern blot analysis, found no gross structural alterations in ER message [10]. Piva *et al.* found that ER mRNA correlated with ER protein [11], Henry *et al.* found that ER messenger RNA assays were more sensitive than ligand binding [12], and May *et al.* studied the ratio of ER protein to mRNA and found that a high ratio correlated with the risk of relapse [13].

The first RNA variant described was by Garcia *et al.*, who used an RNase protection assay and found in 8 of 66 ER-positive tumours a nucleotide mismatch in the B coding region which correlated with low ligand binding [14]. She subsequently found that the mismatch corresponded with a C to T transition at nucleotide 257, resulting in an alanine to valine substitution which removes a *Bbv*I restriction site [15]. In a rather surprising turn of events, Lehrer *et al.* found that 50% of breast cancer patients with the B variant had spontaneous abortions compared with only 10% of patients with wild-type ER [16], and later reported that spontaneous abortions occur only in the B variant ER-positive breast cancer patients and not in the ER-negative or non-breast cancer patients [17]. No explanation for these findings is yet available.

Murphy and Dotzlaw in 1989 using northern hybridisation analyses of breast tumour RNA found a number of smaller size ER mRNA variants resulting from deletions of the hormone binding domain [18]. They prepared a cDNA library from one of these breast cancer biopsy specimens and found 84 unique aminoacids introduced at the exon 3 intron boundary (aminoacid 253) that were L-1 repetitive sequences [19]. These sequences were followed by a stop codon resulting in a truncated 37 kD protein. More recently, these workers reported an ER variant with an insertion of 6 unique aminoacids at the exon 2 intron boundary (aminoacid 214), finally followed by a stop codon for a total of 220 aminoacids [20]. Fuqua *et al.* have screened selected polymerase chain reaction (PCR) amplified fragments from ER mRNA [21] to discover a number of ER variants in clinical breast cancer tissues [22]. They have found base pair insertions, transitions and deletions, and precise deletion (alternative splicing) of exons 3, 5, and 7.

ER VARIANT FUNCTION

Concerning abnormal function, Scott *et al.* using ER gel-retardation assays found that some ER-positive tumours either did not bind or bound weakly to a synthetic oestrogen response element. This decrease in binding was associated with a 50 kD variant dimer or a 50/67 kD heterodimer of wild type plus variant [23].

One can also consider the possibility of active ER in the absence of oestrogen. Zava *et al.* in 1977 was one of the first to speculate about the possibility of biologically active ER without oestrogen [24]. Horwitz and colleagues suggested that perma-

Correspondence to W.L. McGuire.
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nently activated ER might explain the high persistent levels of progesterone receptor in T47D tissue culture cells [25–27]. Sluyser brought a different focus to the problem and hypothesised that mutated or truncated ER might act as an oncogene and stimulate breast cancer growth even without oestrogen [28].

Fuqua *et al.* [22] have directly examined the function of a number of their ER variants in a yeast model system with a reported gene under control of an oestrogen response element. They discovered receptors with outlaw function, consisting of both dominant-positive receptors which activate transcription even in the absence of oestrogen, and dominant-negative receptors which were themselves transcriptionally inactive, but prevented function of normal ER. Future directions should focus in particular on such dominant-positive and dominant-negative ER variants. With regard to positive variants, we would like to know whether they stimulate tumour growth, and secondly, if so, can they be turned off? With regard to dominant-negative variants, we would like to determine whether they can inhibit tumor growth, and if so, can they be turned on?

William L. McGuire
Gary C. Chamness
Suzanne A.W. Fuqua
University of Texas Health Science Center
Division of Medical Oncology
7703 Floyd Curl Drive
San Antonio, Texas 78284-7884, U.S.A.

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