

ABSTRACT

Oestrogen receptor (ER) mutations have been identified for both ER α and ER β in previous studies. The effects of the deletion variants due to splice mutations on clinical parameters, prognosis and treatment were examined in 61 breast carcinoma patients and 13 control samples from elective reduction mammoplasty procedures, respectively. RNA extracted from fine needle aspirates (FNAs) of breast tissue was reverse transcribed and using nested PCR and sequence analysis the presence of these variants elucidated. Using X^2 and Fisher's exact tests their significance with respect to clinical parameters such as tumour size, nodal involvement, stage, presence or absence of metastases, menstrual status and hormone responsiveness was examined. Kaplan-Meier survival analysis was also determined.

The T-47D breast cancer cell line was cloned with two clones being selected for further analysis, namely TCA3 (hormone sensitive) and TCC1 (hormone resistant). These clones were treated for ten passages with oestrogen metabolites, 17- β -oestradiol and oestriol; oestrogen precursors, androstenedione and cholesterol; an anti-oestrogen, 4-hydroxy-tamoxifen; and the aromatase inhibitor aminoglutethimide, respectively. RNA was extracted from the cells initially and after the tenth passage and the ER α and ER β exon profiles were examined using RT-PCR and sequence analysis. After the tenth passage hormone response tests were performed every 24 hours (up to 96 hours) with cell number being determined using the MTT assay.

The results indicate that ER α and ER β variants do not have any affect with respect to menstrual status and nodal involvement (N). Expression of ER α 2 and ER α 4 are required by the mouse monoclonal antibody (DAKO[®] Clone 1D5) in the immunocytochemical assay used for the recognition of the protein in order to assess ER status and therefore show significance. ER α Δ 2 and, contrary to previous investigations, the variant ER α Δ 3 were not found to play a role in tumourigenesis. ER α Δ 5 was observed to be more prevalent in ER α -positive patients and was usually co-expressed with the complete ER α 5 indicating heterodimerization. ER α Δ 5 showed no significance with respect to progression of disease or response to hormone treatment.

An increase in the ratio of ER α Δ 4: wild-type ER α 4 indicated an increase in metastatic potential of diseased tissue. ER α 4 and ER α Δ 4 heterodimers were present in both T-47D clones and after 10 passages the TCA3 clone grown in 10⁻⁸M aminoglutethimide indicated a complete loss of ER α 4 without altering hormone responsiveness. These results suggest that ER α Δ 4 may play a role in progression of disease but not in the acquisition of tamoxifen resistance.

ER α Δ 6 was observed in 15% of patients but not in the T-47D clones or the control samples. An increase in the expression of ER α Δ 6 among patient samples significantly increased their metastatic potential ($p=0.018$). ER α Δ 6 was also observed as significant with respect to stage of disease ($p=0.023$) indicating the possible relevance of ER α Δ 6 in progression of the disease.

ER α Δ 7 was the most frequently observed variant and did not show any significance with regard to any of the clinical parameters examined. The presence of ER α Δ 7 did not show significance with regard to hormone response *in vivo* but *in vitro* the presence of this variant, expressed as a heterodimer with the wild-type ER α 7, conferred greater sensitivity to tamoxifen in the tamoxifen resistant clone TCC1.

Multiple exon deletions of ER α were also observed. The two more significant multiple deletion variants were those involving ER α Δ 4, namely, ER α Δ 2-ER α Δ 6 and ER α Δ 4-ER α Δ 6. The multiple variant ER α Δ 4-ER α Δ 6 may be involved in tumour progression.

ER β variants were not examined in as much detail as ER α variants due to insufficient material available for analysis. The two domains, the DNA binding domain and the ligand binding domain, of ER β were analyzed in a few of the patients and in the T-47D clones. They were not found to be significant with respect to the clinical parameters investigated and the ER β profiles of the TCA3 and TCC1 clones remained unchanged after 10 passages under varying growth conditions.