LACKING EXON5 OF VARIANT ESTROGEN RECEPTOR IN HUMAN BREAST CANCER

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Methods: The target sequence of ER RNA covering exon 4-6 (1082-1520bp) was amplified in 7 clinical human breast cancer tissues by reverse transcription and polymerase chain reaction (RT-PCR) techniques. Results: PCR products were transferred to nitrocellulose membranes and hybridized using a $^32$P-ATP labeled ER 29 oligonucleotide probe representing the antisense strand of the ER cDNA sequence 1271-1299. Specific bands were found at 438 and 300 base pairs in two tumors. The 300 base pair of PCR product was recovered from ER+/PR+ and ER+/PR- tumor, respectively. Conclusion: Dideoxy sequence analysis revealed that they contained the variant ER completely missing exon 5.

Key words: Breast cancer; Estrogen receptor; RT-PCR; Variation

ER is an excellent marker of differentiation. It predicts improved disease free survival in breast cancer and, the most important is to predict the likelihood of benefit from endocrine therapy.1 But 50% of patients fail to respond to hormone despite the presence of ER.2 Recent evidence of foreign countries showed that several variant ER forms had been found in tissues of breast cancer. The variant ERs would lead to altered function and sometimes to hormone resistance.3 In this paper, our objective was to determine whether defects in ER occur in tumors taken directly from patients with breast cancer in our country for further studying on the relationships between ER protein structure and function as well as between ER protein structure and phenotype. The results may make us to find new prognostic factors and formulate the scheme for the treatment of breast cancers more accurately. They may also be fit for other endocrine-dependent tumors except breast cancer.

MATERIALS AND METHODS

Human Tumor Specimens

Frozen human breast tumor specimens, stored at -70°C, were obtained from the surgery of Beijing Institute for Cancer Research (BICR) and consisted of tissue remaining after report assays performed in the pathology laboratory of BICR. All of the tumors containing 1ER-/PR-, 3ER+/PR-, 2ER+/PR+ and 1ER-/PR+ were examined by dextran coated charcoal (DCC) assay.

PSG5-ERcDNA

Recombinant plasmid PSG5-ERcDNA was obtained from Dr. Pierre Chambon (Lab. of Genetic Molecular Eucaryotes, National Scientific Research Center, France).

Reverse Transcription-polymerase Chain Reaction (RT-PCR)
RNA was isolated with RNATRIZOL™ reagent (GibcoBRL) using lg of tumor specimens. RNA integrity was assessed through electrophoresis by denaturing agarose gels containing formaldehyde and concentration was determined by UV absorption prior to reverse transcription and PCR amplification. After an initial denaturation at 94°C for 2 min, 2 units of AMV reverse transcriptase was added, and reverse transcription was allowed to proceed at 42°C for 2 h. The cDNA product was then amplified addition. The ER up-stream primer (nucleotide 1082 base pair to 1102 base pair of human cDNA sequence) was 5′-GGA GAC ATG AGA GCT GCC AAC-3′; the down-stream primer (nucleotide 1501 base pair to 1520 base pair of human cDNA sequence) was 5′-CCA GCA GCA TGT CGA AGA TC-3′. PCR was performed according to following protocols: Denaturation was carried out at 94°C for 4 min first. Then each cycle of amplification consisted of a 1 min of denaturation at 94°C, followed by 1 min of annealing (59°C) and 1 min of extension (72°C) steps. After 30 cycles, the final product was extended for 10 min, then on ice at once.

Southern Blotting and Hybridization

One-tenth of the PCR product was applied to a 1.2% agarose gel electrophoresis, and transferred to nitrocellulose by the method of Southern.4 The nitrocellulose membranes were hybridized with 5′-[r-32P]-ATP labeled 3# oligonucleotide probe (5′-TGA ACC AGC TCC CTG TCT GCC AGG GTG GT-3′) representing the antisense strand of the ERcDNA sequence 1272~1300. Selected PCR products which had been doubted the variant ER were purified from acrylamide for dideoxy sequence analysis.

Dideoxy Sequence Analysis

After PCR, 100µl of the PCR product was loaded onto a 5% polyacrylamide gel. After electrophoresis, the gel was stained in ethidium bromide solution (1 µg/ml) for 40 min. The suspect variant DNA bands were excised under UV illumination. Then the gel was soaked in 100 µl of 0.1×TE overnight at 4°C in a 0.5 ml microcentrifuge tube. Then the supernatant was transferred to a new microcentrifuge tube. The 100 µl of 0.1×TE was added to old tube overnight at 4°C again. The released DNA recovered from twofold supernatant was then analyzed by dideoxy sequencing using fmol PCR cycle sequence kit (Promega). Sequencing primer is the up-stream primer of ER exon4 to exon6.

RESULTS

Denaturing agarose gel containing formaldehydes showed that there were 28S and 18S bands clearly in every RNA extracted. It suggested that the RNA isolated can be the template for RT-PCR. The amplified RT-PCR products were analyzed by gel electrophoresis and hybridization with an internal ER oligonucleotide probe (Fig. 1). No ER-specific PCR products were detected in the ER-/PR- tumors. There are two specific bands in one ER+/PR+ and one ER+/PR- tumor. That is a substantial amount of 438 base pair wild-type ER and a minor component of 300 base pair variant ER products, respectively. The selected bands (300 base pair) were recovered from the agarose gel for dideoxy sequence analysis. The variant also contained the wild-type sequence for exon 4 and 6. Exon 5, however, was precisely missing (Fig. 2). These results suggest that alternative splicing may occur in the hormone binding domain of the ER.

![Fig. 1. Expression of ER transcripts in human breast tumors. cDNA was prepared from PSG5-ER cDNA (4,9) and 7 tumors (1-3, 5-8) and analyzed by electrophoresis and hybridization with an ER-specific oligonucleotide probe.](image1)

![Fig. 2. The nucleotide sequence of RT-PCR amplification using primer#1 and #2 (1082-1520bp, 438bp).](image2)