
Identification of a variant form of the human estrogen receptor with an amino acid replacement

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Estrogen receptor (ER) is a ligand-dependent transcriptional regulator. ER protein has several functionally distinct regions, including a DNA-binding domain in its midportion, a ligand binding domain in the carboxy terminal region, and at least one domain, in the amino terminal region, that influences the magnitude of transcriptional enhancement (1,2). We now report the sequence of a frequently occurring ER genetic variant that carries two point mutations in the region of the gene encoding the amino terminal domain of the protein.

In a previous study (3) using cRNA probes generated from subclones of a human ER cDNA (4) we assayed the mRNA in human tissues that express the gene. Solution hybridization/RNase protection revealed a genetic polymorphism within the mRNA region encoding the amino terminal portion of the protein (3). ER-positive human breast tumors that were heterozygous for the variant gene contained, on average, lower concentrations of ER than tumors obtained from homozygous wild type samples, suggesting that the variant gene may produce less ER or a less stable form of the protein.

To determine the sequence of the ER gene variant region, this portion (from nucleotide 189 to 366 downstream from the initiation codon) (3) was amplified from genomic DNA obtained from three heterozygotes. Taq polymerase-catalyzed DNA amplification (5) made use of two synthetic DNA primers: ER1 5'-CtgcaGCGCAGGTC TACGGTCAGACC-3' and ER2 5'AGCaAGCTtCGGCGGCGGGTGCACT-3'. (Small letters indicate bases included in the primers to generate PstI and HindIII restriction sites at the ends of the amplified products.) Amplified products were treated with restriction enzymes and ligated to pGEM3 plasmid DNA. Bacteria were transformed with these DNAs, and recombinant plasmids from each template were sequenced (6). Sequences were either wild type or contained two point mutations, at nucleotides 257 (C-T transition, changing Ala 86 to Val) and 261 (G-C transition, a silent mutation).

While functional significance of the human ER variant remains to be established, we note that the protein sequence of the wild type human ER between amino acids 80 and 89 is identical to that in rat and mouse ER (7,8). The change at nucleotide 257 in the variant human ER removes a BbvI restriction enzyme site and thus provides a method for detecting the variant ER gene in genomic DNA samples.

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