University of Montana

ScholarWorks at University of Montana

Chemistry and Biochemistry Faculty Publications

Chemistry and Biochemistry

1989

Identification of a Variant Form of the Human Estrogen Receptor with an Amino Acid Replacement

T. Garcia

M. Sanchez

J. L. Cox

P. A. Shaw

J. B. A. Ross
University of Montana - Missoula, sandy.ross@umontana.edu

See next page for additional authors

Follow this and additional works at: https://scholarworks.umt.edu/chem_pubs

Part of the Biochemistry Commons, and the Chemistry Commons

Let us know how access to this document benefits you.

Recommended Citation

Garcia, T.; Sanchez, M.; Cox, J. L.; Shaw, P. A.; Ross, J. B. A.; Lehrer, S.; and Schachter, B., "Identification of a Variant Form of the Human Estrogen Receptor with an Amino Acid Replacement" (1989). *Chemistry and Biochemistry Faculty Publications*. 74.
https://scholarworks.umt.edu/chem_pubs/74

This Article is brought to you for free and open access by the Chemistry and Biochemistry at ScholarWorks at University of Montana. It has been accepted for inclusion in Chemistry and Biochemistry Faculty Publications by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.

Authors

T. Garcia, M. Sanchez, J. L. Cox, P. A. Shaw, J. B. A. Ross, S. Lehrer, and B. Schachter

This article is available at ScholarWorks at University of Montana: https://scholarworks.umt.edu/chem_pubs/74

Volume 17 Number 20 1989 Nucleic Acids Research

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

T.Garcia*, M.Sanchez, J.L.Cox[1], P.A.Shaw[1], J.B.A.Ross[2], S.Lehrer[3] and B.Schachter[1]

Departments of Obstetrics and Gynaecology and Reproductive Science, [1]Cell Biology and Anatomy, [2]Biochemistry and [3]Radiotherapy, Mount Sinai School of Medicine, One Gustave Levy Place, New York, NY 10029, USA
Submitted September 14, 1989 EMBL accession no. X15056

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'AGCaAGCTtCGGCGGCGGGTGCAGT-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.

Acknowledgments: We thank P. Chambon for the hER cDNA and M. Evinger for synthesizing ER2. This work was supported by NIH grants CA45754, DE08174, and HD/GM17542.

*To whom correspondence should be addressed at Roussel Uclaf, 111 rt de Noisy, 93230 Romainville, France

References: 1) Kumar V, Green S, Stack G, Berry M, Jin J-R, Chambon P. (1987) Cell 51:941-951.
2) Kumar V, Chambon P. (1988) Cell 55:145-156.
3) Garcia T, Lehrer S, Bloomer W, Schachter B. (1988) Molec. Endocrinol 2:785-791.
4) Green S, Walter P, Kumar V, Krust A, Bornert J-M, Argos P, Chambon P. (1986) Nature 320:134-139.
5) Sakai RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. (1988) Science 239:487-494.
6) Kraft R, Tardiff J, Krauter KS, Leinwand LA. (1988) Biotechniques 6:544-546.
7) Koike S, Sakai M, Muramatsu M. (1987) Nucl Acids Res 15:2499-2513.
8) White R, Lees JA, Needham M, Ham J, Parker. (1987) Molec Endocrinol 1:735-744.

 ©IRL Press