Monoclonal Antibodies to Estrogen Receptor

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Technology description

Summary

Hybridomas D547, DM75 (Human Estrogen Receptor alpha) UCHI 0912
Antigen:Human estrogen receptor alpha
Antigen Background:The estrogen receptor (ER) is a DNA binding transcription factor that regulates gene expression. Estrogen receptors are members of the steroid/thyroid hormone receptor superfamily of ligand-activated transcription factors. There are two different forms of estrogen receptors, typically referred to as alpha and beta. Hormone activated estrogen receptors form dimers, and can form either homodimers (alpha alpha and beta beta) or heterodimers (alpha beta). The ER genes are both alternatively spliced, and at least three ERalpha and five ERbeta exist. ERs are typically located in the nucleus, but can associate with the cell surface membrane, and are rapidly activated by a number of ligands. ERs contain DNA binding and ligand binding domains and are critically involved in regulating the normal function of reproductive tissues. ER receptor-ligand associations are involved with dissociation from heat shock proteins, receptor dimerization, phosphorylation and the association of the hormone activated receptor with specific regulatory elements in target genes. Estrogen receptors are over-expressed in approximately 70% of breast cancers, and this overexpression has been shown to contribute to tumorigenesis.

Antibody Class:Rat IgG2a
Species Recognized:Human
Hybridoma Production:The E-R used for immunization was isolated from calf uterine nuclei and purified as described (Greene et al, 1977, and Greene et al, 1979). Immunizations were carried out with preparations of nuclear E-R containing 12-20% of the radioactivity expected for a pure steroid-receptor complex consisting of one [3H]estradiol per protein molecule of molecular weight 68,000. The amount of estrophilin administered was estimated on the basis of the radioactivity injected. A 2-month old male Lewis rat was immunized at monthly intervals with a series of three intraperitoneal injections of E-R (36ug each) and finally with an intradermal injection (65ug) at multiple sites on the back. Freund's complete adjuvant was used for the primary immunization and incomplete adjuvant was used for booster injections. Three days prior to removal of the spleen for fusion with myeloma cells, the rat was given an intravenous injection of E-R (110ug) in 1 ml of saline. A partially purified immunoglobulin

fraction was prepared from immune serum by two precipitations from ammonium sulfate, 40% of saturation. Control immunoglobulin was obtained similarly from nonimmunized rats.
Spleen cells were taken from the immunized rat 3 days after the intravenous injection of antigen and fused with myeloma cells according to published procedures. Spleen cells were suspended in Dulbecco's modified medium by using a loose-fitting glass tissue homogenizer; viable cells were then separated from erythrocytes, dead cells, and debris by Ficoll-Hypaque gradient centrifugation. For each fusion, 5 X 107 splenic lymphoid cells and 5 X 10^{6} myeloma cells (P3, NSI, or Sp2/0) were mixed in a 60-mm petri dish, and the dishes were centrifuged (250 X g, 3 min) to produce an adherent monolayer of cells. After aspiration of medium, cell fusion was effected by flooding the dishes with 1 ml of 50% polyethylene glycol 1500 in Dulbecco's modified medium. After 30 sec the cells were washed with two 5-ml portions of the medium; the dishes were then flooded with 5 ml of medium containing 20% fetal calf serum and antibiotics (penicillin plus streptomycin) and incubated overnight at 37'C. The cells were removed by gentle scraping with a sterile rubber syringe plunger and pelleted by centrifugation (50 X g, 5 min). After dispersing each pellet in 30 ml of HAT medium containing antibiotics, 100-Ml aliquots of each cell suspension were pipetted into three 96-well Costar plates (no. 3596) and incubated at 37'C in a humidified 5% CO2 atmosphere. Wells were fed 6-7 days later with an additional 100ul of Dulbecco's modified medium containing 20% fetal calf serum. Wells that contained visible cell clusters (10-21 days after fusion) were assayed for antiestrophilin antibody as they became acidic by using the double-antibody precipitation technique described below.
Monoclonal hybridoma cell lines were obtained by limiting dilution. Antiestrophilin antibody-secreting hybridomas were diluted with Dulbecco's modified medium/20% fetal calf serum, and aliquots containing approximately one cell per 100 ,l of medium were pipetted into 96-well Costar plates previously seeded with 10^{6} irradiated (1200 rad; 1 rad = 0.01 gray) Lewis rat thymocytes per well. Wells containing single clusters of hybridomas (10-21 days after limiting dilution) were assayed for antiestrophilin antibody as described below. Cloned cells were then expanded in suspension culture, ultimately without the aid of thymocytes. Cell lines were stored for future use by freezing in liquid nitrogen at 1-10 X 10^{6} cells per ml in Dulbecco's modified medium/20% fetal calf serum/10% DMSO. Cells were removed from suspension culture by centrifugation for 10 min at 1500 X g. Crude immunoglobulin fractions were prepared from the clarified media by a sequence of two precipitations from ammonium sulfate (40% of saturation). Further purification of Ig was achieved either by chromatography on DEAE-cellulose for IgG or by filtration through Bio-Gel A-1.5 M agarose for IgM. The class and subclass of the monoclonal immunoglobulin produced were determined by Ouchterlony analysis, using antisera specific for u chains and for IgG subclasses.

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