Identification of a Novel Human estrogen Receptor (Delta receptor) and its chromosomal localization by Laser Flashes Femtosecond Spectroscopy

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Abstract: We introduced for the first time in the literature by Laser Flashes Femtosecond Spectroscopy a novel human estrogen receptors. This we defined it as (Delta receptor). It has a molecular weight of approximately 67500 with 695 amino acids. The Delta receptor had a half life of approximately 9-16 hours. It has to some extent antiosterogenic action. It is encoded by a gene localized to chromosome No. 14 in close proximity to the genes related to Alzheimer’s disease. It is located on the same chromosome of estrogen receptor B on chromosome Number 14. This new discovery can explain different action of estrogen in the body and the different actions of antiosterogen (tamoxifen) so a new hypothesis will be addressed for the first time that, there is a receptor had agonistic and antagonistic so this will open a revolution in the field of treatment of malignant osterogenic tumor and in the field hormones replacement therapy. There is a homology between estrogen receptor Delta and estrogen receptor Δ 1 & Δ 2.

Keywords: Delta receptors, estrogen receptor, Laser flashes femtosecond spectroscopy.

1. Introduction

The presence of oestrogen receptors (ER) can be determined either by their high-affinity binding for tritiated oestradiol or by using specific monoclonal antibodies and by laser flashes.

2. Material & Method:

Laser Flashes Femtosecond Spectroscopy (Zewail’s Technique)⁶⁻⁷-Zewail’s technique uses what may be described as the world’s fastest camera. This uses laser flashes of such short duration that we are down to the time scale on which the reaction actually happen-femtoseconds. One femtosecond is equal 0.00000000000001 seconds, which is to a second as a second is to 32 million years. This area of physical chemistry has been named femtochemistry. In femtosecond spectroscopy the original substances are mixed as beams of molecules in a vacuum chamber. An ultrafast laser then injects two pulses: first a powerful pump pulse that strikes the molecule and excites it to a higher energy state. And then a weaker probe pulse at a wavelength chosen to detect the original molecule or an altered form of this. The pump pulse is the starting signal for the reaction while the probe pulse examines what is happening. By varying the time interval between the two pulses it is possible to see how quickly the original molecule is transformed. The new shapes the molecule takes when it is excited- perhaps going through one or more transition states-have spectra that may serve as fingerprints. The time interval between the pulses can be varied simply by causing the probe pulse to make a detour via mirrors.

PCR (8).

Screening of Estrogen receptors A,B,D and non estrogen receptors.

PCR Mapping For Estrogen receptors A,B,D estrogen receptors (9,10).

Fluorescence in situ hybridization For Delta (D) estrogen receptors (11,12) Digital image microscopy (13) Delta (D) estrogen receptors (14) Northern Blot analysis Preparation of isolated granulose cells (14) RT-PCR analysis Delta (D) estrogen receptors (8).

The primer pair used for ERD was AATTCAGATAATCGAGCCCCAG and TTTTCAACATTCTCTTCCTTC, corresponding to nucleotides 974-517 and 801-779 of the human was TAGTGTCATCGGCCAGTTAT and GGGAGCCACACTTCACCAT.

Delta (D) estrogen receptors RNase protection assay (8) (PRA) In situhybridzation(8).
3. Results and Discussion

The Use of well-characterized monoclonal antibodies against the human ER purified from MCF-7 cells in combination with a cDNA expression library in µgtl1, and of synthetic oligonucleotide probes, derived from amino acid sequence of the purified protein, with a µgtl1cDNA library, has allowed us to isolate cDNA clones µOR8, isolated using oligonucleotide probes.

Whenever the in vitro translation products of the MCF-7 poly A RNA were examined either before or after hybrid selection, a weaker additional band of approx. 246 kdalton was observed. The nature of the smaller protein is at present unknown. However, it may correspond on an in vitro degradation product of the larger protein, or to a premature termination of translation, since it was only observed in those denaturing sucrose gradient fractions which yielded the 65 kDalton protein. The 346 kDalton component is not observed after immunoprecipitation of iodinated purified MCF-7 ER.

The amount of information required to code for a protein of 65 kdalton corresponds to an mRNA of approx. 2 kbase since the size of the MCF-7 cell ER fraction of the mRNA should be untranslated majority of eukaryotic mRNAs the most 5 AUG is used to initiate translation. Therefore majority of eukaryotic mRNAs is likely to contain a short 5’ and therefore a very long 3’ untranslated region.

It is likely that the ERD possibly by directly binding the hormone-receptor complex. Therefore the ERD protein may consist of at least two functional domains, the hormone and DNA binding sites.

We have mapped the chromosomal localization of human ERD. Using PCR technique (15,16), we show that the human ERD gene is localized on chromosome 6,14 and using the FISH technique we have mapped ERD 6 14q22-24. To broadly characterize the tissue distribution of human ERD, we have employed a “spot blot” technique to detect the presence of ERA RNA in several human tissues, uterus, ovary, small intestine, large intestine.

In the ovary, the signal is localized to the stroma of the cortex and in blood vessels of the medulla as well as to the granulosa cells.

The analysis of the human ERD gene has shown that it is a very large gene, with the translated exons spanning more than 340 kb.

Using cultured human granulosa cells as show that, the granulose cells in humans contain ERD mRNA. Thus it can be concluded that ERD is likely to play an important role in the regulation of follicular growth and oocyte development.

We have shown that ERD has a relatively (17,18) high affinity for several plant-derived substances with estrogenic activity. It is possible that the human ERD expressed in the gastrointestinal tract is exposed to these compounds via the tract is exposed to these compounds via the diet. For several years it has been claimed that estrogens may protect against colon cancer. Similar claims have also been made for diets containing soy protein, a product rich in phytoestrogens. Estrogens have furthermore been shown to affect calcium uptake in the intestine through a poorly understood mechanism perhaps ERD may mediate some of these effects (19).

Estrogen Receptor D
1- Newly Introduced estrogen receptor.
2- It is localized on long arm of chromosome 6 (similar to Erα) and on chromosomes 14 (Short ARM q22 – q 24) Similar to ERSβ.
3- It is in close PR toxicity to genes related to Alzheimer’s disease and to genes related to vascular endothelial growth factor.
4- It has 1080 amino acids, the summation of ERα Amino acid 595 and ERB 485 it can be cleaved by femtochmistry into2 receptor ERα, ERβ it has half life 16-18 hours.
5- So we speculate that ERD is the native of previous 2 ERα, β

Conclusion
This new discovery can explain different action of estrogen in the body and the different action of anisterogen (tamoxifen) so a new hypothesis will be addressed for the first time that, there is a receptor had agonistic and antagonistic so this will open a revolution in the field of treatment of malignant estrogenic tumor and in the field of hormones replacement therapy.

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