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Cloning of estrogen receptor β isoform M cDNA from human testis

Tomoko SHODA, Shuji HIRATA, Kazuhiko HOSHI

Department of Obstetrics and Gynecology, Yamanashi Medical University

It has been reported that estrogen can directly act on the human sperm and affect their motility. Since sperm is translationally inactive, it is unlikely that these effects are evoked by the modulation of expression of the specific gene by the liganded nuclear estrogen receptor (ER). Therefore, the direct effects of estrogen on the sperm are thought to be "non-genomic" action which is mediated by the "non-genomic" ER whose molecular identity has not been clarified so far. Since some endocrine disrupters have estrogenic action, it is possible that the estrogenic endocrine disrupters may also bind to the "non-genomic" ER and may affect the sperm function. From these points of view, we considered that the study on the “non-genomic" ER in the sperm is important to evaluate the direct effects of estrogen and estrogenic compounds on the sperm function.

In the present study, we attempted to analyze the structure of the ERβ cDNA in the human testis. To do this, the human testicular cDNA library was screened by the human ERβ cDNA probe. Nucleotide sequence of the positive clones revealed the presence of the novel isoform (isoform M) of the ERβ cDNA in the testis. This isoform cDNA has a previously unidentified 5'-sequence on the exons 5-8 of the ERβ cDNA. In order to clarify the genomic origin of the novel 5'-sequence on the exon 5, the human leukocyte genomic library was screened by the sequence. Sequence analysis of the positive genomic clones indicated that this 5'-sequence was derived from the novel independent exon (exon M). Since there was one in-frame ATG codon in the exon M , we considered that the ERβ isoform M protein could be synthesized from the ERβ isoform M cDNA using this ATG as the translation initiation codon. In order to investigate this possibility, the ERβ isoform M cDNA was inserted into the eukaryote expression vector, and 293T cells were transfected by the construct. The SDS-PAGE-Western blot analysis of synthesized protein in the cells indicated that the ERβ isoform M protein was translated from the ATG in the exon M of the ERβ isoform M cDNA.

These results indicate that the novel ERβ isoform M mRNA is present in the human testis, and the ERβ isoform M protein encoded by the mRNA is possibly synthesized in the testis. It should be noted that the ERβ isoform M protein has peculiar structure; the isoform M protein possesses the entire part of the estrogen binding domain, but does not have DNA binding domain. Therefore, we considered that the ERβ isoform M, possibly existed in the testis, might have some relations to the "non-genomic" action of estrogen on the sperm.