Identification of Novel C-Terminally Truncated Estrogen Receptor β Variant Transcripts and Their Distribution in Humans

Hirotaka Ishii, Yujiro Hattori and Hitoshi Ozawa

Department of Anatomy and Neurobiology, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan

Background: The nuclear receptor genes, including estrogen receptor β (ERβ), contain non-conventional internal and terminal exons, and alternative choice of the exons yields multiple mRNA and protein variants with unique structures and functions. However, the genomic structure of the intronic and 3′-downstream regions of the human ERβ gene and the presence of novel ERβ variants with non-conventional sequences have not been re-examined in about 20 years. Therefore, we attempted to re-characterize the structure of the human ERβ gene and identify novel non-conventional exons and distinct splice variants.

Methods: Rapid amplification of cDNA 3′-end and RT-PCR cloning were used to isolate human ERβ mRNA variants from the testis. The identified cDNA sequences were mapped on the human genome assembly. Expression profiles of the variants were assessed by RT-PCR analysis.

Results: We cloned multiple ERβ mRNA variants with novel nucleotide sequences from the testis and identified several alternative splice sites, 3′-elongation of conventional coding exons, and novel terminal exons in the human ERβ gene. The variants encode C-terminally truncated ERβ proteins termed ERβ6, ERβ7, ERβEx. 4, and ERβEx. 6. Furthermore, we identified exon 7-defective forms of ERβ2/βcx, ERβ4, ERβ6, and ERβ7. Subsequently, we noted distinct expression patterns of the variants in human peripheral organs and brain subregions.

Conclusion: This study clarified complicated genomic organization and splicing patterns of the human ERβ gene that contribute to the distinct heterogeneity of human ERβ mRNAs and proteins.

(J Nippon Med Sch 2021; 88: 54–62)

Key words: alternative splicing, ESR2, estrogen receptor β, splice variants

Introduction

Pleiotropic hormones, estrogens, have powerful effects on diverse physiological events in reproductive and non-reproductive organs and are involved in pathophysiological processes such as breast cancer, ischemic stroke, and Alzheimer disease. Estrogen signaling is mediated mainly via activation of nuclear estrogen receptors (ERs) and estrogen receptor α and β (gene symbols: ESR1 and ESR2, respectively).

The ER genes encode ligand-induced nuclear transcription factors that contain distinct functional domains: the N-terminal transactivation function, DNA-binding, hinge, and ligand-binding C-terminal transactivation function domains. The genes consist of several 5′-untranslated exons and eight conventional coding exons. Furthermore, the gene transcripts are subject to complicated alternative splicing. Since the discovery of the full-length ERβ (ERβ1), in 1996, various exon-skipping ERβ variants have been identified. In addition, the human ERβ gene contains multiple non-conventional terminal exons, and alternative choice of the terminal exons generate mRNAs encoding C-terminally truncated ERβ variants. Although the variants themselves lack transcriptional transactivation abilities, they were reported to heterodimerize with full-length ERα and/or ERβ proteins and to modulate trans...
C-Terminally Truncated ERβ Variants

Table 1 Oligonucleotide primers used for 3'-RACE and RT-PCR experiments

| Purpose  | Gene | Exon (s) | Direction | Oligonucleotide sequence (5' to 3') |
|----------|------|----------|-----------|-----------------------------------|
| 3'-RACE  | Universal | Reverse | 5'-GCTGTCAACGGTACGGCATGACAGTG (T)15-3' |
|          |        | Reverse | 5'-GCTGTCAACGGTACGGCATGACAGTG-3' |
|          |        | Reverse | 5'-CCCTACGTAACGGCATGACAGTG-3' |
| ESR2     | 4     | Forward | 5'-AGAGATGTTGGGCTACCCTTG-3' |
|          | 4     | Reverse | 5'-TGATCAGCCACCCAGTGCG-3' |
|          | 5     | Forward | 5'-GGTTAATATGGGGCTGATG-3' |
|          | 6     | Forward | 5'-GGAGTGGGGAATCGTGAGG-3' |
| RT-PCR   | ESR2  | 6     | Forward | 5'-GATGAGGGGAAATGCTAGA-3' |
|          |       | 8     | Reverse | 5'-TTCTAAGGACCCGCAGGTG-3' |
|          |       | 6     | Forward | 5'-GATGAGGGGAAATGCTAGA-3' |
|          | β2    | Reverse | 5'-TTCTTAAAGGGCCACCGAGGTG-3' |
|          | 4, 5  | Forward | 5'-AAGAAGATCCCCGGCTTGT-3' |
|          | β3    | Reverse | 5'-TGGCTTCCCCTCAGATAAAC-3' |
|          | 4, 5  | Forward | 5'-AAGAAGATCCCCGGCTTGT-3' |
|          | β4    | Reverse | 5'-CAATCTTCATTTGCCACA-3' |
|          | 4, 5  | Forward | 5'-AAGAAGATCCCCGGCTTGT-3' |
|          | β5    | Reverse | 5'-CACAATATCCCCAAGCG-3' |
|          | 4, 5  | Forward | 5'-AAGAAGATCCCCGGCTTGT-3' |
|          | β6    | Reverse | 5'-AGGAAGGGGAAACAGGTCTC-3' |
|          | 4, 5  | Forward | 5'-AAGAAGATCCCCGGCTTGT-3' |
|          | β7    | Reverse | 5'-AGGAAGGGGAAACAGGTCTC-3' |
|          | 3     | Forward | 5'-ACGAAGTGGGAATGGTGAAG-3' |
|          | 4L    | Reverse | 5'-GCACAGCTCATGGACCTCTA-3' |
|          | 4, 5  | Forward | 5'-AAGAAGATCCCCGGCTTGT-3' |
|          | 6L    | Reverse | 5'-CGAAGTCCAAAAGGAAACCA-3' |
| GAPDH    | 1     | Forward | 5'-TTCGACAGTCAGCCGCATCTT-3' |
|          | 5     | Reverse | 5'-CCGACAGTCGGCCACCTT-3' |

activation of full-length variants. Several studies have reported that these C-terminally truncated ERβ variants are associated with diseases such as breast cancer, lung cancer, prostate cancer, brain tumor, and cerebral apoplexy. Recently, multiple C-terminally truncated ERα variants have been characterized in humans, mice, and rats. They were generated by alternative choice of novel internal and terminal exons between conventional coding exons and exhibited distinct transcriptional transactivation in transfected cells. These results suggest that there are novel C-terminally truncated ERβ variants generated from novel intronic exons with unique structures and functions in humans. However, the genomic structure of the 3'-region of the human ERβ gene and the presence of novel C-terminally truncated ERβ variants have not been re-examined for about 20 years. Therefore, we decided to re-characterize the structure of the human ERβ gene and identify other human C-terminally truncated ERβ splice variants.

Materials and Methods

Rapid Amplification of cDNA 3'-End (3'-RACE) and RT-PCR

Human total RNAs were purchased from Thermo Fisher Scientific (Waltham, MA, USA) and Takara-Clontech (Shiga, Japan). Information on the total RNAs is presented in our previous report. 3'-RACE was performed as described in our previous studies. Human testis total RNA (Takara-Clontech) was reverse-transcribed by using an adaptor-oligo(dT) primer. Human ERβ 3'-end fragments were amplified by nested PCR using LA Taq polymerase (Takara-Clontech). The cDNAs for RT-PCR were synthesized as described elsewhere. The cDNAs (25 ng/tube) were amplified in three steps by using Blend Taq polymerase (Toyobo, Osaka, Japan), as described in our previous studies. Oligonucleotide primers used in the 3'-RACE and RT-PCR experiments were purchased from Nihon Gene Research Laboratories (Sendai, Japan) and are shown in Table 1. Electrophoresis in agarose gels was used to separate the 3'-RACE and RT-PCR products. The products were stained with ethidium bromide, and the gel images were
The genomic organization of the human ERβ gene is shown schematically. The gene is mapped to 14q23.2-q23.3 in human chromosome 14. The open and filled boxes indicate conventional exons and non-conventional terminal sequences, respectively. The bent arrow, dotted lines, and asterisks symbolize a transcriptional start site, alternative splicing sites, and stop codons, respectively. The inward-facing arrowheads show the locations of primer pairs used for RT-PCR.

Cloning and DNA Sequencing
The electrophoresed amplicons of different sizes were extracted from agarose gels with a Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI, USA) and separately cloned into pGEM-T-Easy vectors (Promega). After the sizes of the cloned products were estimated by direct colony PCR, the clones with differently sized products were selected and DNA-sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). In the RT-PCR experiments, at least three separate clones from each band were DNA-sequenced.

Results
Identification of Novel ERβ mRNA Variants
Unlike the rodent ERβ genes, the human ERβ gene is abundantly expressed in the testis. Therefore, we used 3'-RACE to clone human ERβ variant mRNAs, including novel nucleotide sequences, in the testis. The 3'-RACE fragments were amplified by nested PCR by using forward gene-specific and reverse universal primers and cloned into pGEM-T-Easy vectors. We sequenced the amplicons and identified multiple clones containing novel nucleotide sequences. We then mapped them on the human genome assembly (GRCh38/hg38 human assembly) with the BLAT alignment program and identified several alternative splice sites, 3'-elongation of conventional coding exons, and novel terminal exons. Figure 1 shows the genomic structure of the human ERβ gene schematically. We labelled exons containing novel nucleotide sequences as exons 4, 6, 6, and β7 and named the variants containing the respective sequences as ERβEx. 4, ERβEx. 6, ERβ6, and ERβ7. Their nucleotide sequences are shown in detail in Figure 2. Exons β2, β4, and β6 were generated from alternative choice of splice acceptor sites. Exons 4 and 6 were produced by 3'-elongation of exons 4 and 6, respectively. Exon β7 is a novel non-conventional terminal exon located downstream of exon 8 and contains a putative polyadenylation signal (AATAAA).

Expression and Splicing Patterns of Novel ERβ mRNA Variants in the Testis
Expression and splicing profiles of the C-terminally truncated ERβ variant mRNAs in the testis were analyzed by using RT-PCR with forward primers designed in conventional coding exons and reverse primers in novel terminal sequences (Fig. 3). The locations of the primer pairs are shown in Figure 1. We confirmed expression and splicing profiles of the novel variants in the testis and further observed the presence of exon 7-defective forms in the ERβEx. βcx, ERβ4, ERβ6, and ERβ7 amplicons (named as ERβ2δ7, ERβ4δ7, ERβ6δ7, and ERβ7δ7, respectively).

The open reading frames of the variants were confirmed by RT-PCR cloning and DNA sequencing analysis. The mRNA and potentially encoded protein structures of the ERβ variants are shown in Figure 4. The nucleotide
sequences of human C-terminally truncated ERβ variants were registered to the DDBJ/EMBL/GenBank database. The accession numbers are LC122965 for ERβ2δ7, LC122966 for ERβ4δ7, LC122967 for ERβ6, LC122968 for ERβ6δ7, LC122969 for ERβ7, LC122970 for ERβ7δ7, LC122971 for ERβEx.4, and LC122972 for ERβEx.6. The potentially encoded proteins contained the N-terminal transactivation, DNA-binding, and hinge domains but lacked the 1/3-2/3 C-terminal parts of the ligand-binding domain. Only ERβ1 mRNA encoded the complete ligand-binding/C-terminal transactivation domain.
**Distribution of Novel ERβ mRNA Variant Transcripts**

We analyzed the distribution of ERβ variant mRNAs in human peripheral organs and brain subregions by comprehensive use of RT-PCR (Fig. 5). The ERβ1, ERβ2/βcx, and ERβ5 mRNAs were detected after relatively small numbers of PCR amplification cycles (33 and 34 cycles) and were distributed in a broad range of peripheral organs and brain subregions. The ERβ4, ERβ6, ERβ7, ERβEx. 4L, and ERβEx. 6L products were amplified by using a large number of PCR cycles (38 cycles) and observed in a few organs. The ERβ3 and exon 7-skipping variant amplicons were detected only in the testis.

**Discussion**

Nuclear receptor pre-mRNAs are subject to complicated splicing, which contributes to the heterogeneity of mRNAs and encoded proteins. In particular, alternative splicing in the regions encoding C-termini results in variant proteins lacking most or one part of ligand-binding domains and instead possessing variant-specific C-terminal sequences. Nuclear receptor genes including ERβ contain non-conventional terminal exons, and alternative choice of exons yields mRNAs encoding C-terminally truncated variants.

The pioneering studies of Ogawa et al. and Moore et al. described the presence of several non-conventional terminal sequences and C-terminally truncated ERβ variants (ERβ2/βcx, ERβ3, ERβ4, and ERβ5) in humans. In the current study, we reassessed the structure of the human ERβ gene and identified novel non-conventional exons, C-terminally truncated ERβ variants (ERβ6, ERβ7, ERβEx. 4L, and ERβEx. 6L), and exon 7-skipping variant forms (ERβ2δ7, ERβ4δ7, ERβ6δ7, and ERβ7δ7). The ERβ 2/βcx, ERβ4, and ERβ6 mRNAs are generated by alterna-
C-Terminally Truncated ERβ Variants

Fig. 4  mRNA and protein structures of human C-terminally truncated ERβ variants
The structures of human C-terminally truncated ERβ variant mRNAs (left) and their potentially encoded proteins (right) are represented schematically. The “AUG”s and asterisks in mRNA panels indicate translational initiation and termination sites, respectively.

tive choice of splice acceptor sites in exon β6. The novel sequences of the ERβEx. 4 and ERβEx. 6 variants correspond to the 3’-elongated intronic regions of exons 4 and 6, respectively. In particular, the generation pattern of the ERβEx. 4 variant is similar to those of human, mouse, and rat CTERP-1 variants. Although the human, mouse, and rat ERα genes contain non-conventional internal and terminal exons in intronic regions between coding exons, the non-conventional sequences in the human ERβ gene involve 3’-elongation of conventional exons or are located downstream of a conventional terminal exon (exon 8). Moore et al. reported that the ERβ5
transcript was produced by 3'-elongation of exon 7 and splicing to exon 7. However, later research did not confirm the splicing profile\textsuperscript{22,23}, and we could not detect the pattern in the 3'-RACE and ER\textsubscript{β2}/β\textsubscript{cx} amplicons. Recently, a clone encoding the human ER\textsubscript{β5} protein was registered in the public database (Accession #: AB209620), and the clone corresponds to 1-2-3-4-5-6-7-intron-8. Thus, the ER\textsubscript{β5} variant mRNA results from retention of the intron between exons 7 and 8 rather than from 3'-elongation of exon 7 and splicing to exon \textsubscript{β2}.

The human ER\textsubscript{β1}, ER\textsubscript{β2}/β\textsubscript{cx}, and ER\textsubscript{β5} mRNAs were widely distributed and observed after a relatively small number of PCR amplification cycles (33-34 cycles), whereas the other variants exhibited limited expression and required a large number of PCR cycles for detection. The Δ exon 7 variants were barely detectable, except in the testis. Thus, our RT-PCR results suggest that the ER\textsubscript{β} variants are the predominant isoforms in normal human organs. The human testis exhibited abundant and complicated expression profiles of the ER\textsubscript{β} variants. Recent discovery of the well validated antibody against human and rodent ER\textsubscript{β} proteins indicates that the abundant expression of the ER\textsubscript{β} gene in the adult testis is specific to humans\textsuperscript{10,43}. Thus, the expression profiles of human ER\textsubscript{β} variants imply human-specific modulatory roles in testicular estrogen-signaling pathways.

We deduced that the expression levels of the newly identified variants in normal organs except the testis were lower than those of the ER\textsubscript{β1}, ER\textsubscript{β2}/β\textsubscript{cx}, and ER\textsubscript{β5} variants. Thus, the physiological significance of these variants remains unclear. However, an association of the human ER\textsubscript{β} variants with clinical and pathological conditions has been suggested\textsuperscript{44}. Therefore, future studies should examine the precise roles of the human C-terminally truncated ER\textsubscript{β} variants.

In conclusion, this is the first study to show the genomic organization of the human ER\textsubscript{β} gene and characterize novel structurally diverse ER\textsubscript{β} variants that naturally occur in normal human tissues. Although the physiological and pathophysiological relevance of the C-terminally truncated ER\textsubscript{β} variants is unknown, our find-
ings provide useful and fundamental information for further research on ER variants.

Acknowledgments: This work was supported by JSPS KAKENHI Grants-in-Aid (Grant #: 17K16171, 18K06860, and 18K06879).

Conflict of Interest: None declared

References

1. Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? Endocrinology. 1999;20:358–417.
2. Burnz KA, Korach KS. Estrogen receptors and human disease: an update. Arch Toxicol. 2012;86:1491–504.
3. Koellhoffer EC, McCullough LD. The effects of estrogen in ischemic stroke. Transl Stroke Res. 2013;4:390–401.
4. Li R, Cui J, Shen Y. Brain sex matters: estrogen in cognition and Alzheimer’s disease. Mol Cell Endocrinol. 2014;389:13–21.
5. Mangelsdorf DJ, Thummel C, Beato M, et al. The nuclear receptor superfamily: the second decade. Cell. 1995;83:835–9.
6. Zhao C, Dahlman-Wright K, Gustafsson JA. Estrogen receptor beta: an overview and update. Nucl Recept Signal. 2008;6:e003.
7. Ponglikitmongkol M, Green S, Chambon P. Genomic organization of the human oestrogen receptor gene. EMBO J. 1998;17:3385–8.
8. Enmark E, Pelto-Huikko M, Grandien K, et al. Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. J Clin Endocrinol Metab. 1997;82:4288–95.
9. Kos M, Reid G, Denger S, Gannon F. Minireview: genomic organization of the human ERalpha gene promoter region. Mol Endocrinol. 2001;15:2057–63.
10. Ishii H, Otsuka M, Kanaya M, Higo S, Hattori Y, Ozawa H. Applicability of anti-human estrogen receptor beta antibody PPZ0506 for the immunodetection of rodent estrogen receptor beta proteins. Int J Mol Sci. 2019;20:E6312.
11. Hirata S, Shoda T, Kato J, Hoshi K. Isoform/variant mRNAs for sex steroid hormone receptors in humans. Trends Endocrinol Metab. 2003;14:124–9.
12. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A. 1996;93:5925–30.
13. Mosselman S, Polman J, Dijkstra R. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett. 1996;392:49–53.
14. Lu B, Leygue E, Dotzlav H, Watson PH, Murphy LC, Watson PH. Estrogen receptor-beta mRNA variants in human and murine tissues. Mol Cell Endocrinol. 1998;138:199–203.
15. Vladusic EA, Hornby AE, Guerra-Vladusic FK, Lupu R. Expression of estrogen receptor beta messenger RNA variant in breast cancer. Cancer Res. 1998;58:210–4.
16. Speirs V, Adams JP, Walton DS, Atkin SL. Identification of wild-type and exon 5 deletion variants of estrogen receptor beta in normal human mammary gland. J Clin Endocrinol Metab. 2000;85:1601–5.
17. Pooa I, Abraham J, Baldwin K. Identification of ten exon deleted ERbeta mRNAs in human ovary, breast, uterus and bone tissues: alternate splicing pattern of estrogen receptor beta mRNA is distinct from that of estrogen receptor alpha. FEBS Lett. 2002;516:133–8.
18. Springwald A, Latruch C, Skrzypczak M, Goeree R, Ortman O, Treeck O. Identification of novel transcript variants of estrogen receptor alpha, beta and progesterone receptor gene in human endometrium. Endocrine. 2010;37:415–24.
19. Moore JT, McKee DD, Slentz-Kesler K, et al. Cloning and characterization of human estrogen receptor beta isoforms. Biochem Biophys Res Commun. 1998;247:75–8.
20. Ogawa S, Inoue S, Watanabe T, et al. Molecular cloning and characterization of human estrogen receptor beta2: a potential inhibitor of estrogen action in human. Nucleic Acids Res. 1998;26:3505–12.
21. Peng B, Lu B, Leygue E, Murphy LC. Putative functional characteristics of human estrogen receptor-beta isoforms. J Mol Endocrinol. 2003;30:13–29.
22. Pooa I, Abraham J, Baldwin K, Saunders A, Bhatnagar R. Estrogen receptors beta4 and beta5 are full length functionally distinct ERbeta isoforms: cloning from human ovary and functional characterization. Endocrine. 2005;27:227–38.
23. Leung YK, Mak P, Hassan S, Ho SM. Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling. Proc Natl Acad Sci U S A. 2006;103:13162–7.
24. Leygue E, Dotzlav H, Watson PH, Murphy LC. Expression of estrogen receptor beta1, beta2, and beta5 messenger RNAs in human breast tissue. Cancer Res. 1999;59:1175–9.
25. Fujimura T, Takahashi S, Urano T, et al. Differential expression of estrogen receptor beta isoform (ERbeta) and its C-terminal truncated splice variant ERbetaC as prognostic predictors in human prostatic cancer. Biochem Biophres Res Commun. 2001;289:692–9.
26. Tong D, Schuster E, Seifert M, Czervenka K, Leodolte S, Zeilinger R. Expression of estrogen receptor beta isoforms in human breast cancer tissues and cell lines. Breast Cancer Res Treat. 2002;71:249–55.
27. Li W, Winters A, Poteet E, et al. Involvement of estrogen receptor beta5 in the progression of glioma. Brain Res. 2013;1503:97–107.
28. Liu Z, Liao Y, Tang H, Chen G. The expression of estrogen receptors beta2, 5 identifies and is associated with prognosis in non-small cell lung cancer. Endocrine. 2013;44:517–24.
29. Chantzi NI, Palaiologou M, Stylianidou A, et al. Estrogen receptor beta2 is inversely correlated with Ki-67 in hyperplastic and noninvasive neoplastic breast lesions. J Cancer Res Clin Oncol. 2014;140:1057–66.
30. Huang B, Omoto Y, Iwase H, et al. Differential expression of estrogen receptor alpha, beta1, and beta2 in lobular and ductal breast cancer. Proc Natl Acad Sci U S A. 2014;111:1933–8.
31. Wimberly H, Han G, Pinnaduwage D, et al. ERbeta splice variant expression in four large cohorts of human breast cancer patient tumors. Breast Cancer Res Treat. 2014;146:657–67.
32. Ishii H, Shoda Y, Yomogida K, Hamada T, Sakuma Y. Identification of C-terminally and N-terminally truncated estrogen receptor alpha variants in the mouse. J Steroid Biochem Mol Biol. 2011;124:38–46.
33. Hattori Y, Ishii H, Munetomo A, et al. Human C-terminally truncated ERβ variants resulting from the use of alternative exons in the ligand-binding domain. Mol
34. Ishii H, Hattori Y, Munetomo A, Watanabe H, Sakuma Y, Ozawa H. Characterization of rodent constitutively active estrogen receptor α variants and their constitutive transactivation mechanisms. Gen Comp Endocrinol. 2017;248:16–26.

35. Ishii H, Hattori Y, Ozawa H. Identification of a novel C-terminally truncated estrogen receptor α variant (ERα34) with constitutive transactivation and estrogen receptor antagonist resistance. Mol Cell Endocrinol. 2019;503:110693.

36. Ishii H, Tanaka N, Kobayashi M, Kato M, Sakuma Y. Gene structures, biochemical characterization and distribution of rat melatonin receptors. J Physiol Sci. 2009;59:37–47.

37. Kobayashi M, Ishii H, Sakuma Y. Identification of novel splicing events and post-transcriptional regulation of human estrogen receptor alpha F isoforms. Mol Cell Endocrinol. 2011;333:55–61.

38. Ishii H, Kobayashi M, Sakuma Y. Alternative promoter usage and alternative splicing of the rat estrogen receptor alpha gene generate numerous mRNA variants with distinct 5′-ends. J Steroid Biochem Mol Biol. 2010;118:59–69.

39. Hattori Y, Ishii H, Morita A, Sakuma Y, Ozawa H. Characterization of the fundamental properties of the N-terminal truncation (Δ exon 1) variant of estrogen receptor α in the rat. Gene. 2015;571:117–25.

40. Kent WJ. BLAT--the BLAST-like alignment tool. Genome Res. 2002;12:656–64.

41. van der Vaart M, Schaaf MJ. Naturally occurring C-terminal splice variants of nuclear receptors. Nucl Recept Signal. 2009;7:e007.

42. Swope D, Harrell JC, Mahato D, Korach KS. Genomic structure and identification of a truncated variant message of the mouse estrogen receptor alpha gene. Gene. 2002;294:239–47.

43. Andersson S, Sundberg M, Pristovsek N, et al. Insufficient antibody validation challenges oestrogen receptor beta research. Nat Commun. 2017;8:15840.

44. Taylor SE, Martin-Hirsch PL, Martin FL. Oestrogen receptor splice variants in the pathogenesis of disease. Cancer Lett. 2010;288:133–48.

(Received, November 7, 2019)
(Accepted, February 26, 2020)
(J-STAGE Advance Publication, March 31, 2020)