NOTE

Presence of Alternatively Spliced-Estrogen Receptor mRNA Variants in Normal Human Uterine Endometrium and Endometrial Cancer

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Abstract. Presence of alternatively spliced-estrogen receptor (ER) mRNA variants has been revealed in the breast cancer tissues. The ER variants transcribed from these mRNA variants were supposed to cause changes in the estrogen responsiveness of breast cancer. Although uterine endometrial cancer also has an estrogen-dependent profile, these ER mRNA variants have not yet been reported in the tumor. In the present study, we attempted to detect the exon 7 deletion- (del.7-) and exon 5 deletion (del.5) ER mRNA variants in normal human uterine endometrium (hEM) and uterine endometrial cancer tissue (hEC) by the use of reverse transcription-polymerase chain reaction-Southern blotting (RT-PCR-SB) with the PCR primers: hE4 (forward), hE6 (reverse), and hE8 (reverse), which were located in exons 4, 6, and 8, respectively. Two major products were generated from RNAs of both hEM and hEC with primers hE4 and hE8. The nucleotide sequence of the longer product was identical to exon 4–8 of human ER cDNA, whereas that of the shorter one completely deleted exon 7. Moreover, when the RT-PCR was done with the primers hE4 and hE6, the shorter product lacking exon 5 was detected with the longer one having the same sequence as exon 4–6 of human ER cDNA. Since the RT-PCR-SB with primers hE4 and hE8 produced a very low or undetectable level of the signals corresponding to del.5 ER mRNA variant, the level of del.7 ER mRNA variant seemed to be higher than that of del.5 ER mRNA variant. These results strongly suggested that both del.7- and del.5 ER mRNA variants exist in the normal uterine endometrium as well as in endometrial cancer. The ER variants, possibly expressed in these tissues, may play a physiological and/or pathological role.

Key words: Estrogen receptor mRNA, Variant, Alternative splicing, Human uterine endometrium, Endometrial cancer

(RECENT reports on estrogen receptor (ER) mRNA in clinical breast cancer tissue and breast cancer cell lines have revealed at least five types of alternatively spliced-transcripts [1–10]. These ER mRNA variants lose one exon of the ER gene, exon 2, 3, 4, 5 or 7. Fuqua et al. reported the function of variant receptors encoded by the exon 7 or 5 dele-
far. In the present study, we attempted to detect the del.7- and del.5 ER mRNA variants in hEM and hEC.

Materials and Methods

Tissues

Normal human endometrial tissue was freshly obtained from resected uterus at an operation for uterine leiomyoma. Histological examination revealed that the tissue was proliferative endometrium with no abnormal findings. Tissues from well (G1)-, moderately (G2)- and poorly (G3)-differentiated adenocarcinomas and adenoacanthoma were taken from resected uteri at operations for uterine endometrial cancers, and immediately frozen in liquid nitrogen. Samplings of these tissues were done after the patients’ agreement.

RNA extraction

Total RNA was extracted from tissues according to the procedure of Chirgwin et al. [11] with minor modifications; in brief, tissues were homogenized in 4M guanidine isothiocyanate solution and the total RNA was pelleted through a 5.7M cesium chloride cushion by ultracentrifugation at 35,000 rpm (Beckman, SW50.1 rotor) for 12 h at 20 °C. The total RNA was purified by phenol-chloroform extraction followed by ethanol precipitation, and the RNA concentration was determined by UV absorption.

Reverse transcription (RT)

Total RNA from each tissue was reverse transcribed to synthesize single stranded cDNA. 1.25 ng of total RNA extracted from endometrium was incubated at 42 °C for 60 min with 5 units of RAV-2 reverse transcriptase (Takara, Kyoto, Japan) in a 10 µl reaction volume containing 50 mM Tris-HCl (pH 8.3), 100 mM KCl, 10 mM MgCl2, 10 mM dithiothreitol (DTT), 1 mM each of dNTP and 10 µM random hexadeoxynucleotide primer (Takara).

Polymerase chain reaction (PCR)

A PCR was performed as recommended by Ce-
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The specific activity of the probe was approximately $1.0 - 2.0 \times 10^9 \text{cpm}/\mu\text{gDNA}$. After hybridization, the membrane was washed under stringent conditions. The hybridization signal was analyzed by a Bioimaging Analyzing System, BAS2000 (Fuji Film, Tokyo, Japan).

Nucleotide sequencing

The RT-PCR products, isolated by agarose gel electrophoresis, were subcloned into the Sma I - digested PBS M13 + plasmid (Stratagene, La Jolla, CA, USA). Nucleotide sequences of the products were determined according to the dideoxy method by Sanger et al. [16] with a Sequenase DNA sequencing kit (USB, Cleveland, OH, USA).

RT-PCR blank

In order to examine whether contamination of reagents occurred in the present experiments, distilled water was simultaneously subjected to RT-PCR-SB (RT-PCR blank). But no specific signal was obtained from the RT-PCR blank, indicating no contamination occurred in these experiments.

Results

When the RT-PCR with the primers hE4 and hE8 was carried out on total RNA from hEM and hEC, major products of two molecular sizes were generated (Fig. 2A). The nucleotide sequence of the longer product of 517bp was identical to exon 4-8 of the first cloned human ER cDNA (pOR8) [13, 14] with the exception of one nucleotide substitution; the amplified gene contained Gly-400 (GGG) instead of Val-400 (GTG) in pOR8. Since it had been reported that Gly-400 (GGG) was a "wild type" of human ER cDNA and Val-400 (GTG) in pOR8 was a cloning artifact [17], the substitution did not indicate mutation of the ER gene. The sequence of the shorter one of 333bp also corresponded to the hER cDNA sequence but completely deleted exon 7. Although the minor product was detected between these two major signals in samples from hEM and G1, the nucleotide sequence of the minor product was not analyzed. It was therefore unclear whether the minor products corresponded to the del.5 ER mRNA variant. In order to clarify the existence of the del.5 ER mRNA variant in these tissues, RT-PCR with the primers hE4 and hE6 was further carried out. As shown in Fig. 2B, 279bp and 140bp of the product were generated from hEM and hEC. The nucleotide sequence of the longer product was identical to exon 4-6 of pOR8 with the exception of the nucleotide substitution in the codon 400, but the shorter one consisted of exon 4 and 6 of hER cDNA.

Discussion

The 333bp of the RT-PCR product which completely deleted exon 7 and the 140bp of the product deleting exon 5 indicate that del.7- and del.5 ER mRNA variants exist in hEM as well as in hEC, respectively. Since the RT-PCR-SB with primers
hE4 and hE8 produced the very low or undetectable level of the signals corresponding to del.5 ER mRNA variant, the level of del.7 ER mRNA variant seemed to be higher than that of del.5 ER mRNA variant. Regions other than exon 4-8 of ER mRNA could not be analyzed by the method in the present study, therefore, both 517bp of the product from RT-PCR with hE4/hE8, and 279bp of that with hE4/hE6 might correspond not only to wild type ER mRNA but also to the other ER mRNA variants (for example deletion exon 2 or 3 [2, 3, 9]). Moreover, for the same reason, the 333bp of the RT-PCR-SB signal with primers hE4 and hE8 and the 140bp of the signal with primers hE4 and hE6 could indicate the existence of the multiple exons-deleted ER mRNAs (for example deletion of exon 2 and 7). Further study is necessary to clarify the existence of the other exon deleted ER mRNA variants and the multiple exons-deleted ER mRNAs in the hEM and hEC.

It is well known that human uterine endometrial cancer as well as breast cancer is classified into four subtypes from the view of the status of ER and progesterone receptor (PR): ER positive (+)/PR+; ER+/PR negative (−); ER−/PR+; and ER−/PR− [2, 18, 19]. Because uterine endometrial PR is an estrogen-inducible receptor, the development of hEC which possessed the ER−/PR+ profile had not been clarified. Fuqua et al. reported that the del.5 ER variant encoded by del.5 ER mRNA variant possessed a dominant positive function on wild type ER [1, 6, 7], so that the existence of the del.5 ER mRNA variant in the hEC might explain the development of ER−/PR+ subtype of uterine endometrial cancer. Moreover, the del.7 ER mRNA variant was detected in hEC at a higher level than the del.5 ER mRNA variant. This del.7 ER mRNA variant was reported to encode the del.7 ER variant having a dominant negative function on wild type ER [2, 4]. Although some types of impairment of PR gene expression had been considered to cause ER+/PR− subtype endometrial cancer [20, 21], the del.7 ER variant, possibly expressed in the cancer, might also be involved in the development of the subtype of hEC.

To our surprise, these mRNA variants existed in normal endometrial tissue as well as in endometrial cancer. Because quantitative analysis was not carried out in this study, it was unclear whether the ratio of the level of the del.7- or del.5 ER mRNA variants to that of the wild type ER mRNA was different in normal endometrium from that in endometrial cancer. But the existence of the del.7- and del.5 ER mRNA variants in the normal endo-
metrium suggested that the ER variants, possibly expressed in the normal uterine endometrium, might play a physiological role.

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References


