Report

Incidence of an estrogen receptor polymorphism in breast cancer patients

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Key words: breast cancer, estrogen receptor gene, polymorphism

Summary

We previously identified a polymorphism in the human estrogen receptor (ER) gene, within the coding region for the protein’s amino terminal B-domain. In estrogen receptor-positive (ER+) breast tumors, the variant allele was preferentially associated with lower levels of ER, and was clinically correlated with frequent spontaneous abortions. DNA sequencing revealed a point mutation that changes codon 86 from Ala to Val and a silent mutation in codon 87. Because we initially detected the variant allele by analyzing RNA, only those tissues in which the ER gene is actively expressed were suitable for genotype analysis. We now describe an assay that uses genomic DNA as the substrate for determining the ER B genotype; DNA containing the polymorphic region of the ER gene is amplified by the polymerase chain reaction, then the amplified DNA is hybridized with radiolabeled oligonucleotide probes complementary to the wild type and variant ER alleles. This method allowed us to determine the ER B genotype of women with ER+ and ER- tumors, starting with minute amounts of DNA from frozen or paraffin embedded tissues. ER B genotyping was also performed on women without breast cancer using DNA extracted from blood cells. The combined results from analyses of RNA and DNA from 300 breast cancer patients showed that 12% were heterozygotes. In the ER+ group (n = 183), 11.5% carried the variant gene compared to 12.8% in the ER-negative group (n = 117) (χ² = 0.11; df = 1; p > 0.25). No link to tumor histology could be established. Preliminary data on DNA from blood of healthy women over the age of 50 (n = 64) yielded a slightly lower ER B-variant frequency (9.4%); this frequency was not significantly different than that in the breast cancer groups. Thus, while the variant ER allele is associated with low ER levels in ER positive breast tumors, its frequency is not different in the ER+ and ER- tumor groups and may be unrelated to breast cancer development.

Introduction

A naturally occurring polymorphism in the human estrogen receptor gene was first identified by a RNA/RNA hybridization-nuclease protection assay of estrogen receptor (ER) mRNA in estrogen receptor positive (ER+) breast tumors and non-tumorous hysterectomy samples [1]. The variant
gene contains two nearby point mutations within the protein coding B-region of the receptor [2]. Subsequent analyses of women in the ER+ tumor group revealed that those with the variant gene had a significantly increased incidence of spontaneous abortion [3]. This suggests that the variant ER gene product is associated with altered ER function.

Tumor ER status often serves as a criterion for selecting the mode of post-surgical treatment of breast cancer patients [4]. Also, tumor ER negativity has been associated with lower survival rates among breast cancer patients [4]. In this context, we noted that in the ER+ tumor group, presence of the B-variant allele was correlated with low ER protein levels [1]. We therefore questioned whether the frequency of the variant gene might be greater in the group of women with ER- tumors.

Most frozen breast tumor samples that lacked measurable amounts of ER protein also contained negligible amounts of ER mRNA [1, 5, 6]. This precludes using the assay of RNA for identifying ER-B variants. Therefore, in the current study we report an assay for detecting the ER B-variant genotype that uses genomic DNA rather than RNA as the diagnostic substrate. Specifically, a small segment of DNA around the region of the B-variant is amplified by the polymerase chain reaction (PCR) [7] and the ER B genotype is determined by allele specific oligonucleotide hybridization (ASO) [8].

This assay enabled us to identify ER B-variant women by analyzing DNA from ER- breast tumors. Moreover, the ASO assay of amplified DNA allowed us to determine the ER B genotype of any individual from whom we could obtain a DNA sample. This included individuals whose breast tumors had been preserved in paraffin blocks and individuals who, irrespective of whether they had breast cancer, volunteered to provide blood samples.

**Methods**

**Subjects**

All subjects in the current study were women. Breast tumor samples, both frozen and paraffin embedded specimens, were obtained through the Department of Pathology at Mt. Sinai Hospital. Blood samples from women who did not have breast cancer were obtained in the Department of Obstetrics and Gynecology at Mt. Sinai Hospital and at Long Island Jewish Hospital; these samples were obtained from both clinic and private patients after receiving their informed consent.

**DNA extraction**

One hundred twelve DNA samples were extracted from sections of formaldehyde fixed, paraffin embedded breast tumors that were up to three years old. DNA was isolated using the method of Wright and Manos [10]. In general, three tissue sections were digested in 100 μl of digestion buffer.

In 13 cases, DNA was isolated from the same guanidine isothiocyanate homogenates of frozen tumors previously used to obtain RNA [11]. Here, after ultracentrifugation over a CsCl cushion, the DNA at the interface was recovered and stored at −20°C until needed, then dialysed against 10 mM Tris, 0.1 mM EDTA, pH 8.0 (TE), and further purified by treatment with 50 μg/ml protease K in 100 mM Tris, pH 8.0, 50 mM EDTA, 0.5% SDS at 37°C for 4 hr, extracted with phenol/CHCl3, then CHCl3 alone, then precipitated with ammonium acetate and ethanol. DNA was redissolved in a small volume of TE.

All other DNA samples were prepared from blood cells. Five ml blood were collected into EDTA-containing tubes, and tubes were stored at 4°C for 5 hr to 3 d in an upright position. Serum was discarded and the ‘buffy coat’ interface, along with a small amount of overlying serum and underlying red blood cells, was transferred to a 5 ml capped tube. Tubes were filled with cell lysis buffer (10 mM Tris, pH 7.5, 640 mM sucrose, 5 mM MgCl2, 1% Triton X-100), mixed, then centrifuged for 20 min at 8000 × g, 4°C. Nuclear pellets were recovered and resuspended in 0.45 ml 75 mM NaCl, 25 mM EDTA, pH 8.0, and processed essentially as described above.

DNA isolation was carried out in separate facilities and with separate equipment from those used...