Ovarian Carcinoma-associated TaqI Restriction Fragment Length Polymorphism in Intron G of the Progesterone Receptor Gene Is Due to an Alu Sequence Insertion

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Abstract

Alu sequences, short, repetitive transposable DNA elements, are factors in a number of genetic diseases. We previously identified a germline TaqI RFLP, located in intron G of the human progesterone receptor gene, that showed an association with the incidence of sporadic ovarian carcinoma. Furthermore, the polymorphism was characterized as a small (~300-bp) insertion that was inherited in a Mendelian fashion. Because of its insertional character, we named this polymorphism PROGINS. We report the identification of PROGINS as a 306-bp Alu element of the PV or HS-1 Alu subfamily.

Introduction

The human PR belongs to the steroid-thyroid-retinoic acid receptor superfamily of transcription factors. Upon binding their cognate ligands, these receptors interact with specific enhancer DNA sequences, leading to activation or inhibition of a series of target genes (1). The genomic organization of the human PR gene, located on chromosome 11q22–23 (2), has been described (3). Along with tumor estrogen receptor content (4, 5), PR estimation yields prognostic and diagnostic information in breast (6) and ovarian (7) malignancies, indicating a positive relationship between receptor content and overall survival. It has been suggested that aberrations in the regulation or expression of the human PR gene may underlie the genesis and/or progression of these and other gynecological malignancies (8, 9).

Southern blot TaqI restriction analysis of human leukocyte genomic DNA using a 1.85-kb human PR partial cDNA detected a germline RFLP, located in intron G of the human progesterone receptor gene, that contained an additional TaqI restriction site in intron G, in the ligand-binding domain-encoding region of the gene (10). PCR analysis of intron G of the PR gene, using primers derived from exon 7/intron G and intron G/exon 8 boundaries (3), was consistent with the presence of a small insertion (~300 bp) in intron G of the PR allele (11), named PROGINS. Here we report the identification of PROGINS as a 306-bp, Alu direct repeat element of the PV or HS-1 Alu subfamily.

Materials and Methods

Human leukocyte genomic DNA was extracted from whole peripheral blood samples (10) and analyzed by PCR (11) as described previously. Intron G PCR products from individuals previously typed T1/T1 and T2/T2 by Southern restriction analysis (11) were cloned into the SfiI site of pCR-Script SK(+) (Promega Corp., Madison, WI) and transformed into XL-1-Blue supercompetent cells as recommended by the supplier (Promega). Plasmid DNA from two independent clones was extracted and sequenced using standard procedures. Analysis of the sequence obtained was carried out using the EMBL/GenBank nucleic acid databases Basic Local Alignment Search Tool (BLAST) program and the PC-Gene program.

Results

The PCR products of alleles T1 and T2 (Fig. 1) were cloned and sequenced. Intron G of the T2 allele contained sequence identical to that of T1, with the exception of an Alu direct repeat element inserted 897 bp downstream of the exon 7/intron G boundary. The nucleotide sequence of this element (Fig. 2) showed that the Alu: (a) is inserted in an orientation opposite to that of the PR gene; (b) contains a 25-nucleotide poly(A) tract; and (c) is flanked by 14-mer direct repeats of PR intron G sequence (Fig. 3). Furthermore, the previously identified additional TaqI site of the T2 allele (10) was located. The site of the Alu insertion is in an A/T-rich sequence of the intron, in agreement with preferential sites of Alu integration. Alignment of this Alu sequence with known Alu elements indicated highest sequence match with the PV or HS-1 (12) Alu subfamily, considered the most recently inserted subfamily. Differences were noted between this Alu sequence and the PV/HS-1 consensus (nucleotides underlined in Fig. 2): C→T at position 89, an additional A in the string of As at position 127; C→T at position 138; and G→C at position 269. Our data are consistent with the previously documented polymorphism of members of the PV/HS-1 Alu subfamily in the general population (12).

The primary sequences of the Alu insertion and its flanking regions of human PR intron G sequence were analyzed to locate novel splice branch and acceptor site combinations created by the Alu insertion. A consensus splice acceptor site T(10)TGAG was identified in the Alu insertion (Fig. 3). A consensus splice branch site YNYRAY (where Y = pyrimidine, R = purine, N = any base and A = adenine) was located between nucleotides −53 and −47 relative to the A of the consensus splice acceptor site in the Alu sequence (Fig. 4).

Discussion

We have identified a variant allele of the human PR gene, named PROGINS, containing a 306-bp Alu direct repeat insertion of the PV/HS-1 Alu subfamily in intron G in the hormone-binding domain-encoding region of the gene. Alu elements have been incriminated as factors in the generation of several diseases. Hereditary defects in the low density lipoprotein receptor gene, causing familial hypercholesterolemia, result from deletions or duplications in which Alu repeated
sequences occur at the rearrangement break points (13). De novo insertion of a PV/HS-1 Alu element in the intron separating exons 6 and 7 of the neurofibromatosis-type 1 gene induced aberrant splicing of the primary transcript and resulted in the generation of neurofibromatosis (14).

An initial pilot study established the association of this Alu insertion with sporadic ovarian carcinoma (10). In that study, we speculated that the association of the polymorphism with ovarian carcinoma might reflect a fault in the processing of PR transcripts. Subsequently, the Hardy-Weinberg disequilibrium of the PROGINS genotypes was demonstrated in breast cancer patients (11). Furthermore, recent studies have confirmed an association of the Alu insertion with ovarian carcinoma, and in breast cancer, associations of the Alu insertion with axillary node disease and advanced tumor stage have been demonstrated (15).

Bearing in mind that ovarian carcinomas express multiple PR forms (16), the observations above, along with the data presented in this paper, suggest that the Alu insertion may result in anomalous transcription of the PR gene, either by recombination or by missplicing of the primary transcript. The insertion brings a consensus splice acceptor site into the proximity of an upstream consensus splice branch site, the combination of which potentially directs the encoding of a variant form of PR exon 8. The correct transcription of codons in this exon is essential to the functioning of the human PR; it has been demonstrated that encoding of an alternative exon 8 results in complete loss of both ligand binding and transcriptional activation in response to progesterone (17).

To our knowledge, these are the first reports of the association of an Alu insertion with gynecological malignancies. Recently, loss of heterozygosity studies have identified a tumor suppressor gene common to breast (18), ovarian (19), and cervical (20) malignancies in the chromosomal region 11q22-24. Our data, in conjunction with these studies, suggest that the human PR gene may have a tumor-suppressive role in these diseases.

Acknowledgments

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References

PROGESTERONE RECEPTOR GENE ALU INSERTION


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