

Fragment Analysis of the p53 Gene in Ovarian Tumors

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Introduction

The role of the p53 tumor suppressor gene in human carcinogenesis has been extensively studied. One central function of the p53 protein is control of cellular growth after DNA damage through mechanisms involving growth arrest and apoptosis (1-3). These functions are believed to be at least partially mediated by the ability of p53 to act as a transcription factor. Mutations in the p53 gene are found in most human malignancies and current research is focusing on their role in cancer initiation and progression. p53 gene mutations can lead to defective cellular responses after DNA damage, dysregulated cell growth, and tumor formation. The identification of p53 gene mutations in tumor cells is of diagnostic and therapeutic importance because: (a) tumors with a mutant p53 gene are usually resistant to certain chemotherapeutic agents or radiation; (b) a variety of tumors bearing a mutant p53 gene have a less favorable prognosis than tumors of the same type with a wild-type p53 gene (4).

The frequency of p53 gene mutations is high in cancers of the colon (5), breast (6), lung (7), ovary (8), and brain (9). About 10% of the mutations are deletions or insertions (10). Insertions range from 1 to 14 nucleotides in length and in most cases, the inserted nucleotides duplicate the sequences of the neighboring region. Deletions range from 1 to 37 nucleotides. Presence of deletions/insertions in 6 out of 11 newly established ovarian carcinoma cell lines have been reported (11). In this report, we studied

the presence of deletions and insertions in the p53 gene in 89 primary ovarian tumors.

Materials and methods

TUMOR SPECIMENS

Eighty-nine primary ovarian tumors were collected at the Department of Obstetrics and Gynecology, University Hospital, Turin, Italy. Immediately following surgery, a representative portion of each tumor was selected during quick section procedures in the operating room, snap frozen in liquid nitrogen, and stored at -89° C until analysis. Genomic DNA was extracted with a phenol/chloroform-based procedure (12). The ethanol-precipitated DNA was redissolved in TE buffer (10 mmol/L Tris, pH 7.4, 1 mmol/L EDTA). The extracted DNA was quantified by absorbance measurements at 260/280 nm and kept at 4° C until analysis.

PCR AMPLIFICATION AND FRAGMENT ANALYSIS

The PCR primers and PCR conditions used in this study are described in detail elsewhere (13). Individual PCR reactions were used to amplify each of the 11 p53 exons by using Cy-5 labeled primers. Electrophoresis of PCR amplified fragments were performed on an Automated DNA Sequencer (A.L.F. Express™, Pharmacia, Uppsala, Sweden) as previously described (13). The PCR products for which an abnormality was identified by fragment analysis were electrophoresed on 6% polyacrylamide minigels using Tris-borate/EDTA (TBE) electrophoresis buffer (Novex, San Diego, CA, USA). After staining with ethidium bromide, the PCR products were excised from the gel, DNA was extracted in 10 mmol/L Tris.HCl, pH 7.4 and reamplified by PCR under the same optimized conditions. The reamplified PCR products were additionally used for sequencing.

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Manuscript received February 12, 1998; revised and accepted June 8, 1998.

TABLE 1
p53 Deletions in Ovarian Tumors

Sample	Codons Affected	Predicted Protein Length (Aminoacids)	Exon	Sequence ^a
1	183–185	211	5	TGCTCAGATAGCGATG
2	227, 228	244	7	GCTCTGACTGTA
3	292, 293	303	8	CAAGAAAGGGGA

^aThe deleted nucleotides are underlined and the adjacent repeats are in bold.

AUTOMATED SEQUENCING

The Cy-5 labeled primers used for sequencing exons 5, 7, and 8 are described elsewhere (14) along with nonlabeled PCR primers, which were used to amplify DNA from exons 5, 7, and 8, suitable for direct DNA sequencing. The ThermoSequenase[™] sequencing protocol (Amersham, Life Sciences, Inc., Arlington Heights, IL, USA) was followed according to the manufacturer's instructions. Direct DNA sequencing was performed with the A.L.F. Express[™] DNA sequencer.

Results and discussion

Fragment analysis is a method that detects gene deletions and insertions based on changes in the size of DNA fragments. Length changes of one or more bases can be readily detected but point mutations cannot be identified. Genomic DNA samples extracted from 89 primary ovarian tumors were subjected to PCR amplifications of all 11 exons of the p53 gene and then to fragment analysis. No abnormalities were identified in 61 of the tumors. In 18 tumors, we detected 3 deletions and 15 insertions. All insertions were localized in the PCR product from exon 3 and the 3 deletions in the PCR products from exons 5, 7, and 8. We additionally characterized some of these genetic abnormalities by direct DNA sequencing.

The DNA samples for which an abnormality was detected by fragment analysis were subjected to direct DNA sequencing. The PCR products for exons 5, 7, and 8 were electrophoresed on 6% polyacrylamide gels in order to separate the normal and abnormal amplified fragments that appeared as distinctly moving bands on the gel (homoduplexes and heteroduplexes). Each DNA fragment was then extracted from the polyacrylamide gel, reamplified by PCR, and sequenced. The 3 deletions identified are shown in Table 1.

The 8bp deletion (underlined sequence) in exon 5, codons 183–185, is adjacent to a 3bp repeat sequence (GAT). The deletion affects the reading frame leading to a truncated protein of 211 aminoacids. The 4bp deletion (underlined sequence) in exon 7, codon 227 is adjacent to a 3bp repeat sequence (CTG). This deletion also affects the reading frame leading to a truncated protein of 244 aminoacids. The 4bp deletion (underlined sequence) in exon 8, codon 292 is also adjacent to a 3bp repeat sequence (AAG) and it also affects the reading frame leading to a truncated

protein of 303 aminoacids. A search of the p53 database (<http://perso.curie.fr/tsoussi/>) revealed that the 4bp deletion observed in exon 7 was previously reported (15) while the 8bp deletion in exon 5 and the 4bp in exon 8 have not been reported. The deletions adjacent to duplicated sequences support a slipped-mispairing mechanism which takes place during DNA replication, as proposed by Jago *et al.* (10). The insertions in fragment 3 were not studied further since we previously found that these lie in intronic sequences and represent polymorphism (13).

Acknowledgement

This work was supported by a grant from Visible Genetics Inc., Toronto, Ontario, Canada.

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