Abnormal Structure and Expression of the p53 Gene in Human Ovarian Carcinoma Cell Lines

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ABSTRACT

In an effort to analyze molecular mechanisms of human ovarian carcinogenesis, we studied the structure and expression of the p53 gene in different cell lines established from human ovarian carcinomas. In all six lines (PA-1, Caov-3 and -4, OVCAR-3, SK-OV-3, and Kuramochi), p53 abnormalities were detected. In the SK-OV-3 cell line, Southern analysis suggested the presence of sequence deletions/rearrangements in at least one allele of the p53 gene, and transcripts were not detectable by either Northern or polymerase chain reaction analysis. Sequence analysis of the entire coding region of the p53 gene revealed point mutations resulting in codon changes of a highly conserved region of the protein in four cell lines, Caov-3 and -4, OVCAR-3, and Kuramochi. In the Caov-3 cell line, the point mutation resulted in chain termination at codon 136. Quantitation of p53 protein by immunoprecipitation analysis revealed a 6-fold higher than control cell level in PA-1. By contrast, p53 protein was not detectable in lines Caov-3 and SK-OV-3. We conclude that altered levels of p53 gene expression and/or mutant forms of the p53 gene product are associated with all human ovarian cancer cell tested.

INTRODUCTION

Multiple genetic changes have been noted to occur during carcinogenesis, among them the activation of oncogenes and the inactivation of p53 and other tumor suppressor genes (1, 2). In a wide variety of tumors, the function of the p53 protein appears to be impaired by mutations that have an impact on its synthesis and/or proper assembly (3-15).

Despite their prevalence, tumors of the female reproductive system have only recently become a focus of p53 gene analysis. We and others detected a variety of mechanisms that are responsible for functional p53 inactivation in human uterine carcinoma cell lines (16, 17). Here, we extend our examination to the p53 gene mutations of the ovary. Among many chromosomal abnormalities observed in ovarian carcinomas are those affecting the p5 map of chromosome 17, the genetic locus of p53 (18-20). This prompted us to describe mutations of the p53 gene in cell lines derived from human ovarian carcinomas and to study their effect on gene expression on the RNA and protein level.

MATERIALS AND METHODS

Cell Lines. Six human ovarian carcinoma cell lines (PA-1, Caov-3, Caov-4, OVCAR-3, SK-OV-3, and Kuramochi) were analyzed (21-24), and we used the SiHa cell line as a positive control of immunoprecipitation (17). We obtained the Kuramochi cells from the Japanese Cancer Research Resources Bank; the other cell lines were from the American Type Culture Collection. All cell lines were maintained under conditions recommended by the suppliers.

Southern Blot Analysis. High molecular weight genomic DNA was prepared by a published proteinase K/phenol-chloroform extraction method (25). Human placental DNA was obtained from Oncor, Inc. DNA samples were digested with restriction endonucleases, separated by electrophoresis on 0.8% agarose gels, and transferred to a nylon membrane. The membrane was hybridized with a 32P-labeled p53 probe. The p53 complementary DNA plasmid php53c-1 was kindly supplied by Dr. Moshe Oren (26). The 1.9-kilobase XbaI fragment of php53c-1, containing the entire p53 coding region, was used as a probe. This fragment was labeled with [α-32P]dCTP, using the Random Primer Kit (Stratagene, Inc.).

RNA Isolation and Northern Blot Analysis. Total RNA was extracted from cells by the guanidinium thiocyanate extraction procedure (27). Total placent al RNA was obtained from Clontech, Inc. Samples (20 μg) were denatured with 6.3% formaldehyde and 50% formamide, subjected to electrophoresis on a 1% agarose gel, and transferred to a nylon membrane for hybridization.

Immunoprecipitation. Subconfluent cells were cultured for 1 h in methionine-free medium. Cells were labeled with [35S]methionine at 200 μCi/ml (Amersham; specific activity, >1000 Ci/mmol, 1 Ci = 37 GBq) for 3 h in 10-cm culture dishes. Cells were washed in phosphate-buffered saline and extracted in 1 ml of lysis buffer (1% Nonidet P-40-150 mM NaCl-5 mM EDTA-50 mM Tris/HCl, pH 8.0) containing 1 μM phenylmethylsulfonyl fluoride. Lysates were centrifuged at 4000 rpm for 20 min, and monoclonal antibody PAB 421 (Oncogene Science) was added (28, 29). After 3 h of incubation on ice and a 10-min centrifugation at 2500 rpm, 100 μl of protein A-Sepharose beads (Pharmacia) were added to supernatants. After 1 h incubation at 4°C, beads were pelleted by centrifugation and washed four times in lysis buffer. The immunoprecipitates (100 μg) were directly resuspended in sodium dodecyl sulfate for polyacrylamide gel electrophoresis, using 10% slab gels. After electrophoretic protein separation, gels were processed for autoradiography with Amplify solution (National Diagnostics) according to the manufacturer's instructions. Autoradiography was performed at -70°C.

Cloning and Sequencing. Sequencing of the entire p53 coding region was performed as reported previously (16). Fragments containing the p53 promoter region (30) were amplified by mixing 5' primer (AGG AAA GGA TCC AGC TGT G) and 3' primer (GAA GCG TGT AAA ACG TCG) and 1 μg of genomic DNA, and 2.5 units of Thermus aquaticus polymerase (Cetus) with PCR2 buffer (15 mM MgCl2-500 mM KCl-2 mM deoxynucleotide triphosphate-100 mM Tris/HCl, pH 8.3) in a total volume of 100 μl. PCR product was digested with HindIII and XbaI and subcloned into HindIII-, XbaI-digested PGEM-3Z. More than 100 colonies were used as templates in the sequencing reaction.

RESULTS

Southern Blot Analysis of the p53 Gene. High molecular weight genomic DNA prepared from individual cell lines was digested with restriction enzymes HindIII, PvuII, and BamHI and electrophoresed on a 0.8% agarose gel. Compared to the control, an aberrant restriction pattern was detected only in the SK-OV-3 cell line but not in the other cell lines. After digestion with HindIII, two fragments, 7 and 2.5 kilobases, respectively, were observed in all cell lines. In SK-OV-3 cells, an additional faint band (about 4.4 kilobases) was seen (Fig. 1). Additional faint bands also appeared when SK-OV-3 DNA was digested...
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PCR amplification of the p53 entire coding region resulted in a band of wild-type size in Caov-4, but not in SK-OV-3 samples (Fig. 3).

p53 Protein Levels in Human Ovarian Carcinoma Cell Lines.
p53 protein expression was analyzed using immunoprecipitation. Monoclonal antibody PAb421 that is directed to an epitope near the carboxyl-terminal end of p53 (28, 29) was used for these studies. A specific band of p53 was detected in control cells as reported previously (17). PA-1, Caov-4, OVCAR-3, and Kuramochi cell lines displayed a p53 peptide band of wild-type size, whereas p53 peptide was not detectable in Caov-3 and SK-OV-3 cells (Fig. 4). Compared to the control cell, PA-1 showed about 6-fold overexpression of p53 protein.

Sequence Analysis of the Entire p53 Coding Region. The results are summarized in Table 1. The C to T point mutation with PVUII and BamHI (data not shown). These results suggest partial deletion or rearrangement in at least one allele of SK-OV-3 cells.

Northern Blot Analysis. A human γ-actin probe served as an internal control for possible variations in the amount of RNA loaded from each sample. As seen in Fig. 2, PA-1, Caov-3, OVCAR-3, and Kuramochi cells showed 2.5-kilobase transcripts. In these cell lines there is no overexpression compared to the normal placental p53 mRNA. Transcripts of p53 were not detectable in Caov-4 and SK-OV-3 cell lines. Sequence analysis of promoter regions upstream of exon 1 failed to reveal any explanation for the absence of p53 transcripts in these two cell lines (data not shown). PCR amplification of the p53 entire coding region resulted in a band of wild-type size in Caov-4, but not in SK-OV-3 samples (Fig. 3).

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Our studies emphasize that p53 gene mutations or aberrant expression patterns are frequently associated with cells derived from human ovarian cancer. The SK-OV-3 cell line, the p53 gene was rearranged, and no transcripts or protein products could be detected. There was no obvious defect in the p53 promoter region that could explain the absence of steady state levels of p53 mRNA. In three other cell lines (Caov-4, OVCAR-3, and Kuramochi), we found point mutations in a highly conserved domain of the gene, resulting in single amino acid changes. Two of these occurred at CpG sites that are frequent targets of mutations in a variety of tumors and are attributed to the deamination of methyl cytosine (31, 32). In line Caov-3, a point mutation resulted in a chain termination signal likely to truncate the p53 peptide at amino acid 135. This may serve to explain why the monoclonal antibody PAb 421 antibody that is directed against the carboxy-terminal part of the wild-type p53 peptide failed to detect a protein product corresponding to the mutated gene. Point mutations such as those found in lines Caov-3 and -4, OVCAR-3, and Kuramochi are often, but not always, associated with a loss of the second p53 allele. Our sequencing gels reveal no evidence for the presence of a wild-type allele in these lines, suggesting that wild-type p53 has been lost in the underlying ovarian tumors.

Line PA-1 presents a puzzle that is presently unresolved. The line displayed nearly 6-fold increased levels of p53 protein. There is no evidence to suggest that excessive levels of wild-type p53 protein can provoke malignancy. To the contrary, onco-gene-mediated cell transformation has been shown to be inhibited by increased levels of wild-type p53 (33, 34), and wild-type p53 can completely abolish the tumorigenicity of tumor-derived cells (35). Clearly, our findings with line PA-1 mandate further research into the consequences of wild-type p53 overproduction in specific tissues of the mammalian organism.

The frequent p53 gene abnormalities in human ovarian carcinoma cell lines suggest that inactivation of p53 function may play a significant role in human ovarian carcinogenesis. However, it should be noted that our studies have been performed on cell lines derived from human ovarian carcinomas. Further investigations on primary tumors will be necessary to define the relationship between p53 abnormalities and ovarian carcinogenesis.

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