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Advances in Brief

Ovarian Cancer Genomic Instability Correlates with p53 Frameshift Mutations[1]

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Abstract

We hypothesize that genomic instability plays an important role in causing specific types of p53 mutations in ovarian cancer. To test this hypothesis, 78 tumors were analyzed for p53 mutations with SSCP analysis of the entire open reading frame. At the same time, alterations in 10 microsatellite loci including di-, tri-, and tetranucleotide repeats were evaluated. Fourteen (26%) of all mutations were insertion/deletion mutations. All insertion/deletion mutations were associated with one of the following features: runs of purines or pyrimidines, repeats of short sequences, or palindromes. There was a strong association of generalized microsatellite instability with p53 in contrast to tumors with other types of mutations or wild-type p53 ($P = 0.007$). These characteristic p53 mutations appear to be caused by generalized genomic instability rather than to be the direct cause of genomic instability. These findings suggest the existence of additional novel DNA repair genes important to the carcinogenic process.

Introduction

p53 dysfunction is the most common molecular genetic change associated with many cancers. Jego *et al.* reported that 90% of the mutations in the *p53* gene were single-point mutations for a series of 740 independent mutations sequenced from a variety of human cancers (1). Indeed, for many cancers, a p53 point mutation clearly provides a growth advantage for tumor cells due to loss of the G_1-S checkpoint arrest afforded by wild-type p53 and may allow cells to proliferate in the absence of apoptotic cell death. These functions have earned the *p53* gene product the nickname "guardian of the genome".

Our laboratory has reported recently that insertion/deletion mutations of the *p53* gene occur commonly in ovarian cancer (2). Because these mutations frequently occur at iterated bases and in palindromic or repeat sequences, we and others have postulated that such mutations occur as a result of a slippage mechanism due to a mismatch repair deficit (3). In such cases, p53 insertion/deletion mutations may be caused by genomic instability rather than causal of genomic instability. Our laboratory has also reported that genomic instability is a frequent event in ovarian cancer (4). Thus, we hypothesize that p53 insertion/deletion mutations are associated with generalized genomic instability.

Materials and Methods

Preparation of Tissue. Diagnosis and classification of all tumors were verified by pathology review at our institutional Gynecological Oncology Tumor Board. Ninety-one patients underwent surgery for invasive epithelial ovarian cancer between 1991 and 1995 at the University of Iowa. Tumor samples were obtained from 75% of these patients and snap frozen, whereas paraffin-embedded materials were used on three tumors. DNA isolation and preparation techniques have been reported previously (2). Peripheral blood lymphocytes were obtained from patients as a source of normal DNA (5).

Genomic Instability Analysis. Ten loci containing di-, tri-, and tetranucleotide repeat sequences and representing different chromosomes were obtained from Research Genetics (Huntsville, AL). Microsatellite probes included dinucleotide (D2S123, D3S1611, D10S197, D11S904, D13S175, and NME1), trinucleotide (androgen receptor), and tetranucleotide repeats (DXS981, DXS6800, and DXS6807). Genomic instability was also assessed both by conventional gel analysis of paired germline and tumor DNA and a new method termed *Alu*/AP-PCR.[3] The interrelationship of these methods and technique of *Alu*/AP-PCR are reported elsewhere (4).

Detection of p53 Mutations. Ovarian cancers were screened for mutations in the entire coding sequence of the *p53* gene using PCR and SSCP analysis, as we have reported previously (2). Tumor DNA with suspicious migratory patterns on SSCP analysis was sequenced utilizing intron-based γ-^{32}P-end-labeled primers and the fmol DNA sequencing system (Promega Biotech), as described previously (2, 5). Both strands of the DNA product from the PCR reactions were sequenced to check for fidelity. Abnormalities were verified by resequencing the same region, utilizing products from a replicate PCR reaction to avoid mistaking an early-cycle PCR error as a mutation.

Statistical Analysis. The χ^2 test was used to determine whether a relationship existed between variables utilizing StatGraphics (Statistical Graphics Corp., Rockville, Maryland). $P < 0.05$ was considered statistically significant.

Results

Seventy-eight patients with ovarian cancer were evaluated for genomic instability and p53 mutations. A detectable band shift was noted in 57 tumors by SSCP analysis. Sequence analysis of these samples revealed mutations in 54 tumors (Table 1). Fourteen (26%) were insertion/deletion mutations; 9 were nonsense mutations, including 2 splice-site mutations; and 31 were missense mutations. Most of the specific p53 mutations and their locations have been reported previously (2).

Deletions were observed more frequently than insertions (Table 2). Single- and multiple-bp deletions were observed with similar frequencies (58% single bp *versus* 42% multiple bp, P = not significant). Three of seven single-bp deletions were at an iterated position, and in all cases the bp was iterated more than once. The remaining four single deletions were associated with direct repeats of 2 or 3 bp or palindromic sequences. Five of the 12 deletions were composed of two or more nucleotides. In each case, a direct repeat of 2–5 bp flanked both sides of the deleted segments.

The definition of genomic instability appears to vary between authors depending on number of altered loci. Thus, RER-positive tumors were defined in two alternative ways based on the number of affected loci (Table 3). Microsatellite alterations were found in 29 (37%) ovarian tumors at one or more loci and in 13 (17%) at two or more loci. The most frequent location of MI was at D3S1611 (13%), D13S175 (15%), and NME1 (15%). Table 3 summarizes the associ-

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[3] The abbreviations used are: *Alu*/AP-PCR, *Alu*/arbitrarily primed PCR; SSCP, single-strand conformational polymorphism; RER, replication error; MI, microsatellite instability.

Table 1 *Spectrum of p53 mutations*

p53 mutation	No. (%)
Insertion/deletion	14 (18)
Nonsense	7 (9)
Missense	31 (40)
Splice	2 (3)
None	24 (30)

ation of insertion/deletion mutations with MI. Seventy-one % of tumors with insertion/deletion mutations had MI at one or more loci and 43% at two or more loci. In contrast, tumors with missense, nonsense, or splice site mutations had MI at one or more loci or two or more loci only 35% and 12% of the time, respectively. Tumors with wild-type p53 were associated with MI just 21% of the time. The association of p53 insertion/deletion mutations with MI was highly significant ($P = 0.007$). Four of five tumors (80%) with two or more bp deletions had MI.

Alu/AP-PCR identified genomic instability in 54% of cases. Correlation of *Alu*/AP-PCR findings with microsatellite markers has been presented previously (4). *Alu*/AP-PCR abnormalities were found in 85% of tumors with insertion/deletion mutations compared to 52% in tumors with other mutations ($P = 0.03$).

Discussion

The loss of tumor suppressor function of p53 protein subsequent to a mutation in its coding sequence seems to be a feature common to most cancers, including ovarian cancer. Wild-type *p53* gene product has been shown to play a role in many cellular functions, including cell cycle regulation. The reported abnormalities in p53 have been detected with a variety of techniques, including immunohistochemical staining as a primary screening modality and SSCP screening less frequently. However, certain mutations may not be detectable by immunohistochemical staining, and most studies using SSCP have limited their search to exons 5–8 (3). As shown previously by our laboratory, this strategy can lead to under-reporting of the true frequency of p53-null mutations (2). We routinely perform a complete evaluation of the p53 open reading frame using SSCP analysis with an estimated sensitivity of more than 90% (2). This approach significantly enhances the detection of null mutations, especially insertion/deletion-type mutations.

There has been controversy regarding the association of genomic instability and mutations in tumor suppressor genes such as *p53*. Strickler *et al.* evaluated 40 gastric adenocarcinomas and found no correlation of p53 mutations with genomic instability (6). Mironov *et al.* examined 22 gastric cancer samples and found only 1 tumor with both p53 and microsatellite alterations (7). However, both of these studies only evaluated exons 5–8 of the *p53* gene. Although we found only one deletion mutation in exon 4, we and others have found null mutations to be common to outside exons 5–8 (2, 3). Thus, the reported lack of association between specific p53 alterations and MI may be due to the limited analysis of the *p53* gene.

The high frequency of cancer-associated mutations has led some to suggest that the wild-type *p53* gene product functions as the "guardian" of the genome, whereby this tumor suppressor gene plays a direct role in modulating nucleotide excision repair pathways (8, 9). Recently, Loeb has suggested that widespread genomic instability associated with cancer cells leads to a cascade of mutations (10). Some of these mutations enable the cancer cell to bypass host regulatory processes. Microsatellites contain multiple repetitive DNA elements, usually located in noncoding regions of the human genome, and are stably inherited under normal conditions. MI reflects widespread genomic instability and is characteristic of cancers arising in families with the hereditary nonpolyposis colorectal cancer syndrome (11, 12). Subsequent studies have revealed that mutations in one of at least four genes, *hMSH1*, *hMLH1*, *hPMS1*, and *hPMS2*, responsible for mismatch repair in cancer cells, may result in a mutator phenotype. Clearly, other genes are involved in this process as well (13).

Jego *et al.* evaluated 740 published p53 mutations and found insertions/deletions in 10% of the cases (1). These authors demonstrated that most insertion/deletion mutations were localized at iterated nucleotides or associated with direct repeats, suggesting that these mutations occur via a slippage mechanism in the course of DNA replication. Greenblatt *et al.* reported that most p53 deletions and insertions are associated with monotonic base runs, adjacent or nonadjacent repeats of short tandem sequences, palindromes, and runs of purines or pyrimidines (3). The ovarian cancer p53 insertion/deletion mutations we have reported are consistent with this model. Fifty % were associated with direct repeat sequences ranging from 2 to 5 bp. Four (29%) insertions/deletions were associated with iterated sequences, and three (21%) were associated with short palindromic sequences. All of these structural characteristics are consistent with DNA strand slippage. This model is further strengthened by the association of a high incidence of MI in tumors with insertion/deletion mutations (71%) compared to tumors with other types of mutations (35%) or no mutations (21%).

Ours is not the first report of the association of MI with insertion/deletion mutations of an expressed gene. Eshleman *et al.* reported a similar finding in the *hprt* gene (14). Huang *et al.* found a statistically significant correlation of adenomatous polyposis coli frameshift mutations with mismatch repair deficiency (15). This pattern of insertion/deletion mutagenesis associated with the open reading frame of p53 appears then to be the result rather than the cause of genomic instability in a sizable fraction of ovarian cancer. Because a family history compatible with the hereditary nonpolyposis colorectal cancer phenotype is distinctly uncommon for our population of ovarian cancer probands (2), our findings led us to postulate the existence of one or more additional DNA repair genes distinct from *hMLH1*, *hMSH2*, *hPMS1*, and *hPMS2*. We are actively engaged in a search for such an additional DNA repair gene at this time.

Table 2 *p53 deletion/insertion mutations*

Tumor	Nucleotide[a]	Exon	Sequence[b]	RER[c]
Deletions				
31[d]	12139	4	CCCCGCGTGGCCCC	0
198[d]	13111	5	GTGCAGCTGTGGGTTGATTCCACA CCCCCGCCCGGCACCCG	1
131[d]	13207	5	GCGCTGCCCCCA	1
88[d]	13231	5	CAGATAGCGA	2
61	13331	6	CCCCTCCTCA	0
81[d]	14010	7	TCTGACTGTA	1
3	14049	7	GTTCCTGC	2
202[d]	14049	7	GTTCCTGC	3
20[d]	14075	7	GAGGCCCATCCTCACCATC	7
279	14490	8	CGTGTTTGTGCCTG	0
84[d]	14566	8	AGCTGCCCCC	2
159[d]	14571	8	CCCCCAGGG	0
Insertions				
213[d]	13362	6	AATTTTGCGT	2
197	13392	6	AACAACTTTT	1

[a] Nucleotide where the deletion or insertion starts.
[b] Deletion and insertion mutation sites are underlined.
[c] Number of altered microsatellite loci.
[d] Previously reported mutations (2).

Table 3 *Relation between p53 mutations and microsatellite instability in ovarian cancer patients*

p53 mutation	RER$^-$ ($n = 49$)	RER$^+$ (one or more loci) ($n = 29$)	P	RER$^+$ (two or more loci) ($n = 13$)	P
Insertion/deletion	4	10		6	
Other	26	14	0.007	5	0.01
None	19	5		2	

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