Distribution of p53 mutations in esophageal and gastric carcinomas and the relationship with p53 expression

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Abstract. Mutations of p53, a tumor suppressor gene, are known to be involved in the pathogenesis of a number of neoplasms. This study investigated the distribution of p53 mutations within both esophageal and gastric adenocarcinomas. The correlation between p53 mutations and an overexpression of p53, which has been reported by other researchers, was also explored. Samples were taken from 17 patients following a surgical resection of the tumor. The patients included 8 cases of adenocarcinoma from the cardia (esophagogastric junction) and 9 cases of gastric carcinoma. Two or three samples were taken from each tumor, plus samples of normal tissue from the patient. Denaturing high pressure liquid chromatography (DHPLC) was employed to detect p53 mutations, and samples found to have mutations were then sequenced. The expression of p53 was determined by immunohistochemistry. DHPLC demonstrated that 37.5% (3/8) of esophageal carcinomas and 44.4% (4/9) of gastric carcinomas have p53 mutations. DNA sequencing showed the same mutation to be present in all of the samples from each tumor, while the corresponding normal tissue was free from mutations (except for 2 cases of polymorphism). The results of immunohistochemistry did not demonstrate a relationship between p53 mutations and the expression of p53 protein, and only 4 of the 7 tumors with p53 mutations showed a positive result. These findings support the hypothesis that p53 mutations are homogeneous throughout a tumor and may thus be a more useful diagnostic and prognostic indicator than the expression of p53, which does not reliably correlate with p53 mutations.

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Introduction

Adenocarcinomas of the esophagus and gastric cardia have demonstrated rapidly increasing incidence rates (1). The incidence of adenocarcinoma in the distal esophagus and proximal stomach are rising more rapidly than any other visceral malignancy in the Western industrial world. More than half of all such cases are advanced carcinomas at the time of initial diagnosis, which are associated with a poor 5-year survival rate (2).

The p53 gene mutation represents one of the most frequently altered tumor suppressor genes in human cancers (3). p53 mutations have been extensively studied in many tumor types in order to establish the diagnostic, prognostic and therapeutic characteristics of the tumors in which they are associated. A mutation of the p53 gene is a crucial factor in the carcinogenesis of many tumor types (4). Hyperdysplastic epithelial cells in the cardia have also been found to have a high frequency in p53 gene mutations.

It is necessary to determine the nature of the mutation in tumor cells, and demonstrate that this mutation occurs homogeneously throughout the tumor tissue, which is an important factor in the characterization of a tumor and selection of the optimal treatment regime. The aim of this study was to investigate p53 gene mutations using several samples obtained from the same tumor specimen. The denaturing high pressure liquid chromatography (DHPLC) technique was used to detect p53 gene mutations in the entire coding region for p53 from exons 5 to 8, the region in which most mutations occur (5). DHPLC is a relatively new technique that uses heteroduplex formations between wild-type and mutated DNA standards to identify mutations. The heteroduplex molecules are separated from homoduplex molecules by ion-pair reverse-phase liquid chromatography on a specialized column matrix, with partial heat denaturation of the DNA strands. DNA sequencing of the mutated gene was performed, and immunohistochemistry was employed to identify any overexpression of the P53 protein.

The aim of this study is to test the hypothesis that the same pattern of mutation occurs throughout the cells of an individual tumor and explore whether such a pattern has any relationship with the expression of p53.

Table I. Clinicopathologic data and mutation of p53 gene in 17 patients.

Case no.	Age	Sex	Organ	T	N	M	G	Stage	Lauren	DHPLC	Samples
1	68	M	Stomach	3	2	0	3	IIIB	Diffuse	-	3 tumors, 1 meta, 1 normal
2	42	M	AEGI	2a	1	0	3	II	Intestinal	+	2 tumors, 1 meta, 1 normal
3	72	M	AEGI	3	1	0	3	IIIA	Mixed	-	3 tumors, 1 meta, 1 normal
4	67	F	Stomach	4	2	1	3	IV	Diffuse	-	3 tumors, 1 normal
5	65	F	Stomach	2	1	1	3	IV	Diffuse	-	3 tumors, 1 normal
6	67	M	AEGI	3	0	0	3	II	Mixed	+	3 tumors, 1 normal
7	69	M	AEGII	1sm	0	0	3	IA	Intestinal	-	2 tumors, 1 normal
8	81	F	Stomach	1sm	0	0	2	IA	Intestinal	+	3 tumors, 1 normal
9	70	M	AEGI	1sm	1	0	2	IB	Intestinal	+a	3 tumors, 1 meta, 1 normal
10	74	M	Stomach	3	2	1	3	IV	Diffuse	-	3 tumors, 1 meta, 1 normal
11	69	M	Stomach	3	2	1	3	IV	Diffuse	+	3 tumors, 1 normal
12	79	F	Stomach	1sm	1	0	3	IB	Intestinal	+	2 tumors, 1 normal
13	76	M	Stomach	1m	0	0	1	IA	Intestinal	-	3 tumors, 3 meta, 1 normal
14	61	M	AEGII	2	0	0	3	IB	Mixed	+	3 tumors, 1 normal
15	49	M	AEGIII	2	2	0	3	IIIA	Intestinal	-	3 tumors, 1 normal
16	57	F	Stomach	3	2	1	3	IV	Intestinal	-	2 tumors, 1 meta, 1 normal
17	66	M	AEGIII	4	1	0	2	IV	Intestinal	-	3 tumors, 1 meta, 1 normal

Tumors, tissue of tumor; meta, metastasis; +a, tumor with a double-point mutation.

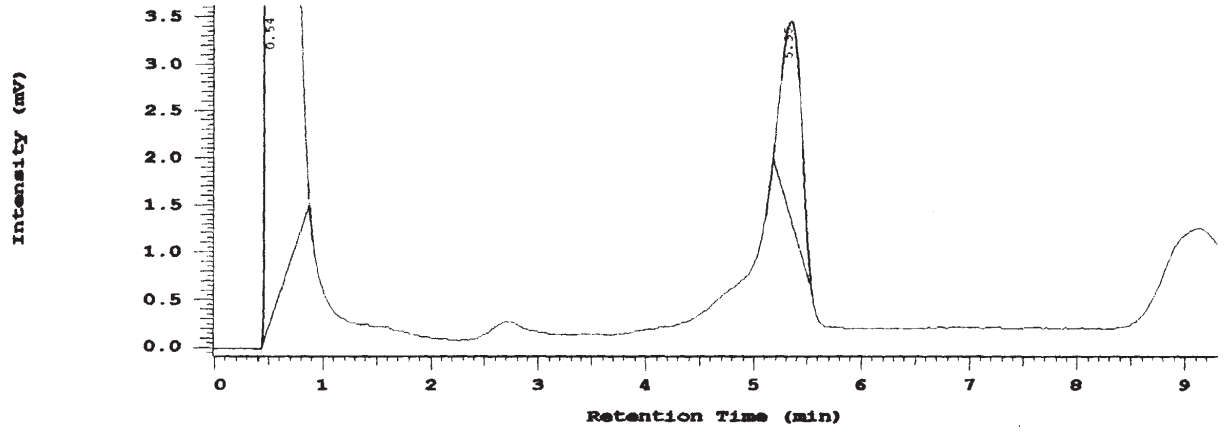

Figure 1. Example of the mutations found in a DHPLC analysis, normal tissue. A normal pattern shows only one peak.

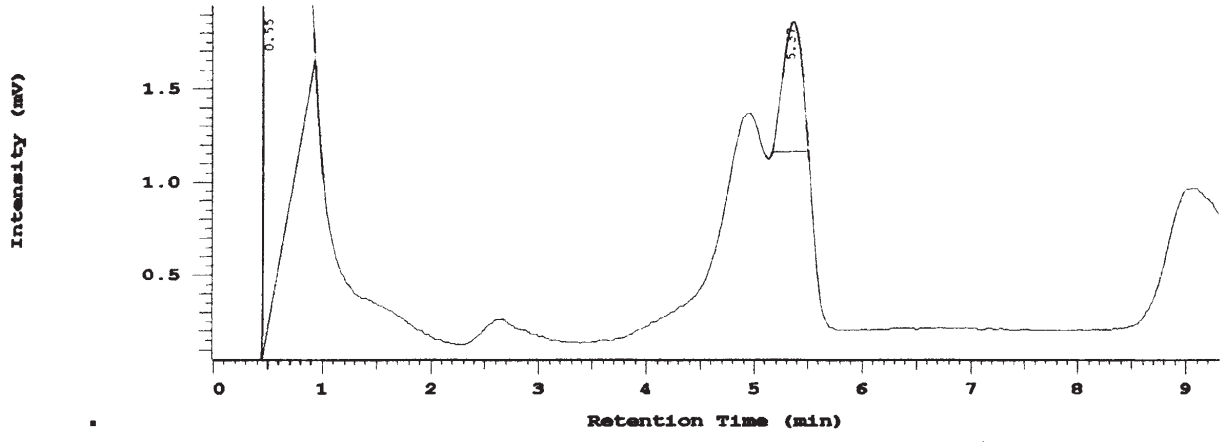

Figure 2. Example of the mutations found in a DHPLC analysis. A mutation pattern shows two peaks.

Table II. Summary of samples with a p53 mutation and result of immunohistochemistry of p53.

Case no.	Exon no.	Codon no.	Nucleotide (amino acid) change		IHC
2	8	Intron 7	Deletion 1 bp		Positive
6	6	208	GTC (Val) →	GAC (Asp)	Positive
8	5	213	TAC (Tgr) →	TAG (Stop)	Positive
9	6	213	CGA (Arg) →	CGG (Arg)	Positive
	7	248	CGG (Arg) →	TGG (Trp)	
11	5	181	CGC (Arg) →	CAC (His)	Negative
12	6	213	CGA (Arg) →	CGG (Arg)	Negative
14	6	214/215	Deletion 2 bp		Negative

IHC, immunohistochemistry; bp, base pair.

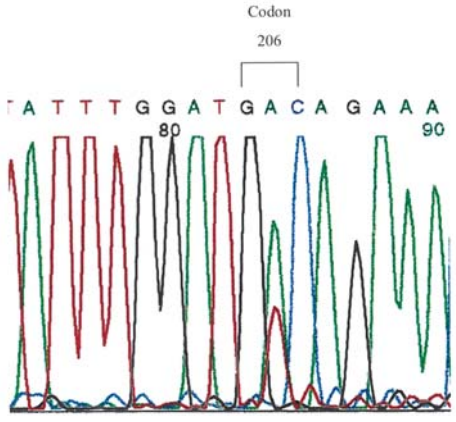

Figure 3. Example of DNA sequencing in the p53 gene at exon 6 with the mutation at codon 208-Val to Asp.

Materials and methods

Patients and tumors. The tissue samples were collected from 17 patients who underwent a curative resection of a primary esophageal or gastric carcinoma in the Surgical Department of the Technische Universitaet Muenchen, Munich, Germany, between September 1999 and January 2000. There were 8 cardia carcinomas [4 adenocarcinoma of the esophagogastric (AEG) junction type I, 2 AEG type II, and 2 AEG type III), and 9 gastric carcinomas. Two or three tumor tissue specimens were prepared from the central and peripheral regions of the tumor, and metastatic lymph node tissue was added in some N(+) cases. Normal tissue specimens were also prepared for each case. The tumors were classified according to the criteria of Lauren (6). Among the cardia and gastric carcinomas, 9 were intestinal, 3 were mixed and 5 were diffuse type. The classification and tumor staging were based on the criteria of the UICC (7). The gastric cancer cases were distributed into classes IA, IB, II, IIIA, IIIB and IV, with the number of cases per class: 3, 3, 2, 2, 1 and 6, respectively. The male to female ratio was 12:5 (70.6%:29.4%), and the average age of all patients was 66.6±10.0 years, with a range of 42 to 81 years. The patient data are summarized in Table I.

DNA isolation and amplification, DHPLC analysis and DNA sequencing. DNA was isolated from paraffin-embedded tumors using standard procedures, as previously described (8). Exons 5-8 of the samples, including the intron/exon boundaries, were amplified by PCR, analyzed by DHPLC and DNA sequencing. PCR amplification, DHPLC analysis and DNA sequencing have also been described in previous studies (8).

Immunohistochemical analysis (IHC). Immunohistochemistry for the p53 protein was performed according to the previously described protocol (9). The p53 reaction was scored as positive when >10% of tumor cells showed nuclear staining. A positive control section of colon carcinoma with mutated p53 was included with each series and was consistently positive.

Results

DHPLC analysis. Mutations of the p53 gene were detected in 21 lesions from 7 patients (Table I). Two cases had a mutation in exon 5, and two patients in exon 6. For exons 7 and 8, there was one patient per exon with a mutation. One case had mutations in both exons 6 and 7. In all cases, no mutations were detected in any normal tissue. In 11 gastric carcinoma (including 2 AEG III cases) and 6 cardia carcinoma cases (4 AEG I and 2 AEG II), 3 and 4 cases with mutations were detected, respectively. In the gastric carcinoma group, two mutations were found in exon 5 and one mutation in exon 6. In the cardia carcinoma group, there was one mutation in exon 8, one in exon 7 and three in exon 6. Of the 9 metastatic lymph node tissue samples examined, evidence of a mutation was observed in two cases. The DHPLC analysis of these metastatic lymph nodes showed the same pattern as the original tumor tissue with mutations in the same exon and same codon. Ten cases did not have a detectable mutation in any of the samples. Figs. 1 and 2 show the normal and mutation patterns for comparison purposes.

Sequencing. For cases showing a p53 mutation based on DHPLC analysis, the nature of the mutation was then explored by DNA sequencing. As a result, all of the mutated tumor samples were found to have the same nucleotide change in all cases (Table II). A nucleotide change was observed in the normal tissue specimens from two cases with a mutation in exon 6. This occurred in codon 213, and was most likely a polymorphism. In two cases in which the DHPLC analysis did not detect any mutations, there were also no mutations found by DNA sequencing. Fig. 3 shows the results of DNA sequencing of the p53 gene.

IHC. Immunohistochemistry, to detect the expression of p53, was analyzed for the 7 cases that had been shown to have mutations in their tumor tissue. Four patient samples were positive; in each of these 4 cases, all tumor tissue and metastatic tissue specimens were positive, while normal tissue specimens were negative. In three cases, all specimens produced a negative reaction. From these results, there appears to be no correlation between the p53 gene mutations and a p53 positive reaction based on immunohistochemistry findings.

Discussion

The presence of p53 gene mutations is one category that can be used to characterize a tumor and a potentially useful predictor of malignant behavior. Such mutations occur as early events during the malignant transformation of Barrett's esophagus (4). Adenocarcinomas of the esophagus and cardia have a frequency and spectrum similar to those of p53 gene mutations, suggesting that these tumors have a common pathogenesis (10). In this study, p53 mutations were detected in 37.5% (3 of 8) of the esophageal adenocarcinomas, and 44.4% (4 of 9) of gastric adenocarcinomas. This compares with the frequency of mutations that have been reported previously; p53 mutations were detected in 55-70% (4,11) (12,13) and 35-63% (11,14,15) of esophageal and gastric adenocarcinoma, respectively. With a sample of only 17 cases, the frequency of such mutations may not be statistically significant, but our findings correlate with those of other studies, particularly for gastric adenocarcinoma.

A positive immunohistochemistry result for p53 expression in a tumor specimen is associated with poor prognosis (16); the 5-year survival rate for patients with a p53 mutation has been shown to be worse than that for those without a mutation (17). Generally, patients with mutations tend to be younger, have signs of more advanced disease, and have a poorer prognosis than patients without such mutations (10).

Adenocarcinoma arising in the gastric cardia might be related to esophageal adenocarcinoma (10,18). It appears that the p53 mutational status is a valuable parameter to identify low-risk (p53 mutation-negative) and high-risk (p53 mutation-positive) groups for treatment failure after curative resections (12). In the upper intestinal region, Fricke *et al* reported a relationship between immunohistochemistry, p53 gene mutation and the accumulation of p53 in gastric cancer (8). If this was confirmed, it could potentially result in a faster screening method, in which numerous samples could be examined simultaneously using mass-screening techniques, i.e. for biopsy specimens. However, the results of this study found no relationship between p53 gene mutations and p53 expression. These results suggest that the p53 mutational status appears to be a more useful indicator than immunohistochemistry when characterizing the malignant properties of the tumor.

In conclusion, the results of this study support the hypothesis that the same mutation is present in all cells throughout the tumor when a p53 gene mutation occurs, but are absent in normal tissue from the same patient. This has implications for the characterization of a malignant tumor from a biopsy sample, as a sample from any region of the tumor will show homogeneity in the p53 gene mutation. This is important for rapid diagnosis, prognosis and the correct choice of therapeutic regime. This study did not establish a relationship between p53 mutations and the expression of p53 in tumors. This is contrary to the findings of other studies and warrants further research.

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