Chromosomal Instability Rather Than p53 Mutation Is Associated with Response to Neoadjuvant Cisplatin-based Chemotherapy in Gastric Carcinoma

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ABSTRACT

Purpose: The objective of the study was to evaluate microsatellite alterations [microsatellite instability (MSI) and loss of heterozygosity (LOH)] and mutation in the p53 gene in relation to response and patient survival to a cisplatin-based neoadjuvant chemotherapy in gastric cancer.

Experimental Design: Fifty-three pretherapeutic gastric carcinoma biopsies were analyzed with 11 microsatellite markers. The entire coding region of the p53 gene (exons 2–11) was analyzed for mutations by denaturing high-pressure liquid chromatography and sequencing. p53 protein expression was evaluated by immunohistochemistry. Patients were treated with a cisplatin-based, neoadjuvant chemotherapy regimen. Therapy response was evaluated by computed tomography scan, endoscopy, and endoluminal ultrasound. The median follow-up of the patients was 45.6 months.

Results: p53 mutations were identified in 19 of the 53 (36%) analyzed tumors. No significant association with response or survival was found for p53 mutation or for p53 protein expression. MSI (either high-grade MSI or low-grade MSI) did not show a correlation with response. With respect to LOH, LOH at chromosome 17p13 showed a significant association with therapy response ($P = 0.002$) but did not reach statistical significance in terms of patient survival. The global LOH rate, expressed as fractional allelic loss (FAL), was assessed, and tumors were classified into tumors with a high (>0.5), medium (>0.25–0.5), and low (0–0.25) FAL value. A statistically significant association of FAL with therapy response was found ($P = 0.003$), with a high FAL being related to therapy response. The sensitivity, specificity, positive predictive value, and negative predictive value for FAL > 0.5 were 45%, 93%, 82%, and 72%, respectively.

Conclusions: A high level of chromosomal instability (high FAL value) defines a subset of patients who are more likely to benefit from cisplatin-based neoadjuvant chemotherapy. p53 mutation status is not significantly associated with therapy response and is not a useful marker for response prediction.

INTRODUCTION

Preoperative (neoadjuvant) chemotherapy for patients with locally advanced gastric cancer is an approach that has been studied since 1989. It is still not a generally accepted concept for clinical practice and has only been used within Phase II studies with various treatment protocols. Only two randomized trials of neoadjuvant chemotherapy have been published thus far, and the overall results were inconclusive (1, 2). However, a consistent finding is that patients who respond to neoadjuvant therapy have a significantly prolonged survival compared with nonresponding patients (3). However, because only 30–40% of patients are responders, the majority of patients undergo several months of toxic, expensive therapy without survival benefit. Unfortunately, there is no reliable, prospectively tested assay for response prediction for gastric carcinoma.

Several molecular markers had been investigated as potential response predictors. Thymidylate synthase is the target enzyme for 5-FU (4), and its expression has been shown to be significantly associated with response to 5-FU-based therapy in gastric carcinoma (4–6). In addition, the expression of ERCC1, an enzyme involved in nucleotide excision repair, has been found to have a significant association with response in a neoadjuvant therapy regimen based on 5-FU and cisplatin (6). Other markers such as glutathione S-transferase, thymidine phosphorylase, vascular endothelial growth factor, and apoptosis-related genes and gene products such as bcl-2, bax, and p53 have mostly been studied by immunohistochemistry, and the results have been inconclusive, so that no marker has been found to be clinically relevant at present (7–10). In a previous study, we found an association between a high rate of LOH, designated

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3 The abbreviations used are: 5-FU, 5-fluorouracil; MSI, microsatellite instability; MSI-H, high-grade MSI; MSI-L, low-grade MSI; LOH, loss of heterozygosity; FAL, fractional allelic loss; DHPLC, denaturing high-pressure liquid chromatography; PPV, positive predictive value; NPV, negative predictive value.
as FAL, and therapy response in patients treated with preoperative cisplatin-based chemotherapy. The chromosomal region of the p53 gene on chromosome 17p13 was among the loci most frequently altered (11). Based on these findings and the known hypothesis that p53 is important for the maintenance of chromosomal stability (12, 13), one could expect an association between p53 mutation and response.

Thus, the goal of our present study was to clarify the role of p53 in relation to therapy response by performing a comprehensive mutation analysis of the whole coding region (exon 2–11) of the p53 gene. To our knowledge, this is the first study that has reported p53 gene mutation analysis with respect to neoadjuvant treatment in gastric carcinoma. In addition, we evaluated our previous findings of an association of a high rate of LOH with therapy response in a larger number of cases.

MATERIALS AND METHODS

Patient Characteristics. In this retrospective study, we analyzed the pretherapeutic biopsies of 53 patients with locally advanced gastric cancer who were treated with cisplatin-based neoadjuvant chemotherapy. Criteria for the inclusion of these patients were the availability of nontumor and tumor tissue from biopsies before treatment and the suitability of cases for the isolation of DNA from tumor areas containing at least 50% tumor cells by manual microdissection. Clinically, the patients must have received at least 50% of the projected dose of the chemotherapeutic regimen. Inclusion criteria for the chemotherapy were as follows: (a) tumor category cT3 or 4, cN0, cM0, and M1 lymph; (b) fitness for cisplatin-based chemotherapy and subsequent surgery; and (c) age, 18–70 years. Exclusion criteria were as follows: (a) confirmed peritoneal carcinomatosis or other distant metastases (M1) except M1 lymph; (b) episodes of severe tumor hemorrhage; (c) clinically evident, severe gastric outlet syndrome; (d) WHO performance status > 1; (e) prior gastric surgery; (f) second malignancies; (g) prior radiotherapy or chemotherapy; and (h) pregnancy.

Pretherapeutic staging to confirm locally advanced adenocarcinoma consisted of upper gastrointestinal endoscopy with endoluminal ultrasound to determine tumor infiltration of the gastric wall (T-category), diagnostic laparoscopy to exclude peritoneal carcinomatosis, and computed tomography scan. The biopsies were graded and classified according to the WHO and Lauren classification (14). Patient and tumor data are shown in Table 1.

All patients were treated within one of three Phase II trails with a polychemotherapy regimen based on cisplatin. The detailed chemotherapy protocols and the number of patients included are shown in Table 1.

Forty-nine of the 53 patients (92%) had a tumor resection after chemotherapy. Four patients were not operated upon due to tumor progression. Based on the resection specimen, 39 patients (80%) had complete resections [International Union against Cancer (UICC) R0], 8 patients had microscopic residual disease (R1), and 2 patients had macroscopic residual disease (R2). All 53 patients were evaluable for response (none were lost to follow-up) and were included in this study. At present, 21 patients are still alive with a median follow-up of 45.6 months (range, 20.4–103.6 months) and a median survival of 34.4 months.

Response Evaluation. Response evaluation was performed clinically as described previously (11). In brief, response was defined as at least 50% reduction in the size of the primary tumor as measured by computed tomography scan, endoluminal ultrasound, and total flattening of the gastric wall in endoscopy. Tumor reduction of <50% or newly detected metastatic lesions were classified as “non-responding.” There were 21 (40%) responders and 32 (60%) nonresponders (Table 1). The responders had a median survival of 76.5 months versus 20.1 months for nonresponders (P < 0.001).

DNA Isolation and Microsatellite Analysis. Paired tumor and nontumor DNA samples from pretherapeutic biopsy specimens were isolated from formalin-fixed, paraffin-embedded tissue after microdissection as described previously (15). Tumor DNA was only isolated from areas containing at least 50% tumor cells. Microsatellite analysis included the same 11 microsatellite markers recently published, and the PCR reactions were essentially performed as described previously (11). The markers were selected because of their location at or near chromosomal sites (3p, 5q, 7q, 11q, 16p, 17p, and 18q) of known or putative tumor suppressor genes involved in gastric carcinogenesis or at sites that are commonly used for the determination of MSI, as we have described previously in detail (11). In addition, some of them have been used to provide a prognostic index in gastric and colon cancer (16, 17). The PCR products were labeled with fluorescence-tagged primers and separated and detected using an automated sequencing system (ABI 377; Perkin-Elmer, Branchburg, NJ).

Scoring of MSI. An additional band in tumor DNA compared with the nontumor sample was classified as instability for this marker. Instability in 5 or more of the 11 markers was...
defined as MSI-H, and instability in 1–4 markers was defined as MSI-L. MSI-L was confirmed by a second PCR.

**Scoring of LOH and FAL.** LOH was determined using the calculation method described by Beckmann et al. (18). If the allele peak ratio was <60%, the tumor was considered to have LOH, representing a signal reduction of at least 40%. This allelic imbalance was defined as LOH, although we could not completely exclude that in some cases the change in allele peak ratio may have resulted from allelic amplification. BAT 40 was homozygotic or ambiguous with respect to heterozygosity and was not evaluated for LOH. Tumors with MSI at a given locus were not evaluated for LOH at that locus. FAL was defined as the ratio of the number of chromosomal sites showing LOH divided by the number of informative chromosomal sites for each case. For the two markers on chromosome 7q and 8q, the combined information was used. The tumors were categorized as low (0–0.25), medium (>0.25–0.50), and high FAL (>0.5) as described previously (11). Tumors with MSI-H were excluded from evaluation for FAL.

**Definition of Sensitivity, Specificity, PPV, and NPV.** The sensitivity of a high FAL value (>0.5) to predict the response to therapy was defined as the proportion of patients with high FAL responding to therapy in relation to the total number of responding patients. Specificity was defined as the proportion of patients with low or medium FAL not responding to therapy in relation to the total number of nonresponding patients. The PPV of high FAL was defined as the proportion of patients with high FAL responding to therapy in relation to the total number of patients demonstrating high FAL. The NPV of high FAL was defined as the proportion of patients demonstrating a medium or low FAL value in their tumors who did not respond to therapy in relation to the total number of patients demonstrating medium or low FAL in their tumors.

**p53 Mutation Analysis.** p53 mutation analysis was performed by DHPLC (Wave System; Transgenomic, Omaha, NE) of the PCR products of exons 2–11. Primers and conditions for PCR and DHPLC analysis of exons 2–5 have been described previously (19). For exons 2–4 and 9–11, the following primers were used for PCR: exon 2-F, tcctctgtcaaggcaagct; exon 2-R, tgcctcttaaggctacca; exon 3-F, gggaagagaaaaattcgagg; exon 3-R, tcaatatgtcggggaca; exon 4-F, gactgtctttctcactac; exon 4-R, cagcccctcagggcaactga; exon 9-F, aagcaagcaggacaa-gaagc; exon 9-R, ccacttgataagaggtccca; exon 10-F, ctcaggtact-ctcagaatatac; exon 10-R, aatggaatctatgtttc; exon 11-F, ccctctctctactgatgtgctc; and exon 11-R, ccaaaaccccaaatggeacg. The PCR reactions were performed in 50 μl of a reaction mixture consisting of 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.0, 1.5, or 2.0 mM MgCl₂; 0.01% gelatin; 200 μM dNTPs and 0.4 μM of each primer. After an initial denaturation step at 94°C for 4 min, 40 cycles were performed consisting of 30 s at 55–65°C and 30 s at 72°C, followed by a final extension of 7 min at 72°C. The PCR products were denatured for 4 min at 94°C and cooled to room temperature at a rate of 1°C/min. PCR products (2–3 μl) were applied to a preheated reverse phase column (DNA-Sep; Transgenomic). Elution of the DNA was performed in a linear acetonitrile gradient of buffers A and B. Buffer A consisted of 0.1 M triethylammonium acetate, and buffer B consisted of 0.1 M triethylammonium acetate and 25% acetonitrile. The optimal temperatures for resolution of hetero-}

duplex and homoduplex DNA for exons 5–8 have been described previously (19). The DHPLC analysis conditions were established for exons 2–4 and 9–11, and the following temperatures and acetonitrile gradients (% buffer B) were used: (a) exon 2, 61°C, 48–59% buffer B and 63°C, 46–56% buffer B; (b) exon 3, 61°C, 51–61% buffer B; (c) exon 4, 63°C, 52–64% buffer B and 66°C, 46–58% buffer B; (d) exon 9, 60°C, 48–59% buffer B; (e) exon 10, 62°C, 50–60% buffer B; 64°C, 49–59% buffer B; (f) exon 11: 61°C, 49–59% buffer B.

**DNA Sequence Analysis.** PCR products with an aberrant DHPLC chromatogram were directly sequenced in both directions starting from a new PCR product. Purification of the PCR product was performed by the Qiaquick gel extraction kit (Qiagen, Hilden, Germany) followed by cycle sequencing with fluorescence-labeled dye terminators and separation with an automated sequence system (ABI 377; Perkin-Elmer). All tumors demonstrating the same mutation were sequenced three times starting from a new PCR reaction to exclude any cross-contamination between tumor samples.

**Immunohistochemistry.** p53 immunohistochemistry was performed on pretherapeutic biopsies using an automated stainer (TeckMate 500; DAKO Corp., Glostrup, Denmark) with the DO7 monoclonal antibody (DAKO) used at a 1:300 dilution after microwave treatment of formalin-fixed, paraffin-embedded 6-μm tissue sections (1 × 20 min, microwave at 750 W, and citrate buffer solution at pH 6.0). The reaction was developed with the streptavidin-biotin-alkaline phosphatase system with fast red used as the reaction indicator. A positive reaction for p53 protein was seen as a nuclear stain, and a case was considered positive for overexpression when >10% of the tumor cell nuclei showed definite staining. Positive and negative controls were included with each staining series. Among the 53 tumors, sufficient material for immunohistochemical analysis and evaluation was available in 48 cases.

**Statistical Analysis.** Medians and ranges were reported for continuous variables, and absolute and relative frequencies were reported for categorical variables. Fisher’s exact test (two-tailed) was used to compare relative frequencies because one or more expected cell counts were frequently less than 5. All survival data were calculated from the date of first chemotherapy to date of death or most recent follow-up. The survival rate was determined by the Kaplan-Meier method. Differences between the survival curves were calculated with the log-rank test. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**p53 Mutation Analysis**

Twenty p53 mutations were identified in the pretherapeutic tumor biopsies of 19 (36%) patients among the 53 analyzed cases. One patient had two mutations (exons 5 and 8). There were a total of six mutations in exon 5, seven mutations in exon 7, six mutations in exon 8, and one mutation in intron 9. Of the 20 mutations, 15 were missense mutations, 3 were frameshift mutations, and 1 was a splice site mutation. Examples of an aberrant DHPLC pattern and the corresponding DNA sequence analysis are shown in Fig. 1. There was no significant correlation of p53 mutations with response. Of the 21 responders, 9 (43%) patients had p53 mutations, compared with 10 patients...
with mutations among the 32 (31%) nonresponders ($P = 0.56$; Fig. 2A). There was also no significant survival difference between patients with or without \(p53\) mutation [median survival with \(p53\) mutation (37.5 months) \textit{versus} median survival without \(p53\) mutation (31.8 months), $P = 0.77$; Fig. 2B]. In Table 2, the \(p53\) mutations are shown in comparison to response.

### \(p53\) Immunohistochemistry

\(p53\) overexpression was found in 17 of the 48 (35%) tumors. No correlation with response or survival was found. Six of 19 (32%) responders and 11 of 29 (38%) nonresponders showed \(p53\) overexpression ($P = 0.76$; Fig. 2A). In the study as a whole, positive \(p53\) protein expression was more frequently found among tumors with \(p53\) mutations because 9 of 17 (53%) cases with \(p53\) mutations were also positive by immunohistochemistry compared with 8 of 31 (26%) cases that were positive by immunohistochemistry among the tumors without \(p53\) mutation, but this difference was not statistically significant ($P = 0.113$). Considering only missense mutations, 8 of 13 (62%) cases with missense mutations showed positive \(p53\) protein expression, compared with 9 of 35 (26%) tumors that were positive among the tumors without missense mutation ($P = 0.039$).

### Microsatellite Analysis

**MSI.** MSI was found in at least 1 of the 11 tested markers in 13 of the 53 (25%) tumors, with 3 (6%) tumors exhibiting MSI-H, and 10 (19%) tumors exhibiting MSI-L. There was no correlation between MSI and response or survival. MSI-H was found in 1 of 21 (5%) of the responding tumors and 2 of 32 (6%) of the nonresponding tumors. MSI-L was seen in 4 of 21 (19%) of the responding tumors and 6 of 32 (19%) of the nonresponding tumors.

**LOH.** LOH at chromosome 17p13 was found in 15 of 45 (33%) informative tumors. There was a statistically significant association between LOH at 17p13 and response, with 10 of 18 (56%) informative responders demonstrating LOH compared with 5 of 27 (19%) nonresponders ($P = 0.022$; Fig. 2A). Despite this significant correlation, there was no significant survival benefit for patients with LOH at 17p13; the 15 patients with a LOH had median survival of 77 months compared with 32 months for the 30 patients without LOH ($P = 0.16$; Fig. 2C). Results at the other chromosomal loci are shown in Table 3. No other marker showed a significant correlation between LOH and response or survival.

In the study as a whole, \(p53\) mutations were observed statistically more frequently in tumors demonstrating LOH at chromosome 17p13; 9 of 15 (60%) informative cases with LOH had a \(p53\) mutation, whereas 6 of 25 (24%) informative cases without LOH had a \(p53\) mutation ($P = 0.017$).

**FAL.** Chromosomal instability as characterized by FAL was significantly associated with response. Among the 20 responding patients, 9 (45%) showed a high FAL ($>0.5$), 2 (10%) had a medium FAL ($0.25$–$0.5$), and 9 (45%) had a low FAL ($<0.25$), whereas among the 30 nonresponding patients, 2 (7%) showed a high FAL, 9 (30%) had a medium FAL, and 19 (63%) had a low FAL ($P = 0.003$; Fig. 3A). The median survival for each of the three groups (FAL = 0.25–0.5, FAL $>0.25$–0.5, and FAL $>0.5$) was 23.9, 34.4, and 76.5 months, respectively, but a statistical difference could not be shown due to the low numbers of cases in two groups (Fig. 3B). In the study as a whole, there was a statistically significant association between
chromosomal instability and mutations of p53. Among the 11 cases with a high FAL value, 7 (64%) had a p53 mutation, among the 11 cases with medium FAL, 7 (64%) had a p53 mutation, and among the 28 cases with a low FAL value, 4 (14%) had a p53 mutation ($P = 0.001$).

**Sensitivity and Specificity of FAL Value.** To evaluate the clinical impact of FAL, we calculated the sensitivity, specificity, and PPV and NPV for response for a FAL value $\geq 0.5$. The sensitivity was 45%, the specificity was 93%, the PPV was 82%, and the NPV was 72%.

**DISCUSSION**

LOH has been considered as a hallmark of many human cancers and may reflect numerical or structural chromosomal abnormalities. The ratio of LOH-positive markers to total informative markers, designated FAL, is considered to be a measure of chromosomal instability. The result of our present study encompassing 53 gastric carcinomas demonstrates a significant association of a high FAL rate with response to neoadjuvant cisplatin-based treatment in gastric cancer and confirms our previous findings based on the analysis of 37 tumors (11). The degree of accumulated allele loss has been shown to be of prognostic value in various cancer types including gastric cancer, and a high degree of tumor LOH has been shown to be associated with tumor aggressiveness and a worse prognosis (16, 17, 20, 21). In light of these findings, it is surprising that the high FAL tumors in our study showed a higher rate of response to a cisplatin-based neoadjuvant treatment. However, genetic alterations that predict a poor prognosis in untreated tumors may also serve as markers of better response in the setting of chemotherapy. Cisplatin supposedly directly damages DNA (22), whereas 5-FU is an inhibitor of thymidylate synthase, a key enzyme for nucleotide metabolism, thereby secondarily affecting DNA and RNA structure (23). Although the underlying mechanism of chemotherapeutic tumor response in our study cannot be exactly explained, our findings that a high FAL value is associated with better response and support the hypothesis of Cahill et al. (24), which states that there is a threshold for genetic instability that, when exceeded (as, for example, by additional DNA damage), results in cell death.

The chromosomal locus that demonstrated LOH most frequently and also showed an association with therapy response in our study was chromosome 17p13. Fifty-six percent of responders and 19% of nonresponders demonstrated an LOH at 17p13. The overall rate of 33% of LOH at 17p13 was in the range (20–60%) found in previous studies of gastric carcinoma (15, 25, 26). Because p53 is located at this locus and is thought to suppress chromosomal instability (12, 13), we were interested in a detailed characterization of p53 mutation status in relation to therapy response and analyzed the entire p53 coding region for mutations. Overall, 36% of cases demonstrated p53 mutations, corresponding to previous gastric carcinoma studies reporting rates ranging from 25% to 63% (27–29). Forty-three percent of tumors in the responding group and 32% of tumors in the nonresponding group exhibited mutations, a difference that was not statistically significant. Similar results were obtained by analyzing the protein expression by immunohistochemistry (32% in responders, 38% in nonresponders).

Among its many roles, the p53 gene product has been suspected to play a major role with respect to chemotherapy response because it is important for the regulation of transcription, DNA repair, and cell cycle control, as well as for the induction of apoptosis (30). However, numerous studies in different tumor types and cell lines have found conflicting results with respect to p53 and chemotherapy response (31–35). In gastric cancer, several studies have demonstrated an association of p53 expression evaluated by immunohistochemistry and therapy resistance (10, 36, 37). We found no such relationship, and the reason for this discrepancy may be related to differences...
in methodology and evaluation of p53 expression or differences in the chemotherapy regimen used. Thus, it has been demonstrated that, when analyzing human colon cancer cell lines that are isogenic with respect to p53 status, p53-deficient cells are more resistant to 5-FU but more sensitive to DNA-damaging agents such as Adriamycin (38). Furthermore, differences in respect to response evaluation may contribute to conflicting results.

The lack of association of p53 mutation in our study may also be due in part to the different effects of p53 mutations, which may range from simple loss of function to dominant negative activity or increased oncogenic activity (39–41). Specific types of mutations that disrupt the L2 or L3 loop domains of the p53 protein are associated with poor response to monotherapy with doxorubicin in breast carcinoma (35). Nine of the p53 mutations we found in our study affected these domains, but we saw no association with response (4 of 21 responders, 5 of 32 nonresponders). However, it is difficult to make definite conclusions about the effect of specific mutations based on our study because the numbers are too small. Overall, the complex situation involving the various functions of the p53 protein, the effects of different p53 mutations, and the different mechanisms of action of the chemotherapeutic agents as well as the biological differences among the analyzed tumors may explain the lack of an association between p53 mutation and response or survival in the present study.

To our knowledge, this is the first report of mutation analysis of the entire coding region of the p53 gene in gastric cancer. An analysis of all studies in the literature that screened the entire p53 gene in other tumor types revealed that 13.6% of the mutations are located outside of exons 5–8 (42). Although the preferential investigation of exons 5–8 in most studies supposedly leads to a considerable bias, we found only 1 mutation outside of exons 5–8. This mutation was a base substitution t>c at nucleotide 993+2 in intron 9 affecting the highly conserved splice consensus sequence at the splice donor site that is suspected to lead to a deletion of exon 9 (43).

In the study as a whole, we found a significant association of the occurrence of missense mutation and p53 overexpression. This is in line with previous observations in various tumors
contribute to the association of LOH with therapy response. It is our findings indicate that other mechanisms unrelated to p53 classic mechanism for tumor suppressor gene deactivation, but p53 tumor suppressor gene by somatic mutation and concomitant LOH is the and not with informative tumors that did not demonstrate LOH (24%). An tumors exhibiting LOH at chromosome 17p13 (60%) than in tumors with a low FAL value (14%). Others have reported the concurrence of p53 mutations with genetic instability [i.e., aneuploidy (12, 13)], which is in line with our findings. However, recent studies of isogenic colorectal cell lines have demonstrated that there is no causal relationship between the concurrence of p53 mutation and chromosomal instability. This, in addition, could explain the lack of a clear association of p53 mutation with response in our study, and it suggests that factors besides p53 are important for the maintenance of genetic stability. Given the numerous genes that are involved in the correct chromosome segregation and maintenance of chromosomal stability, the global characterization of chromosomal instability may be more important for response prediction than the specific characterization of a single genetic factor.

With respect to MSI, we found only a low number of tumors (n = 3) exhibiting the MSI-H phenotype. This low frequency may be related to the relatively high number of proximal-located gastric cancers in our study, whereas MSI-H has been shown to be preferentially found in distal gastric tumors (15, 46, 47). Cell lines that showed the MSI-H phenotype and were defective in mismatch repair systems have been shown to be resistant to treatment by cisplatin (48). However, in our study, among the three patients with MSI-H, one was a responder, and two were nonresponders, which does not support the observation of the cell lines.

In conclusion, our data show that p53 mutation status is not a useful marker for response prediction in gastric cancer. However, our data show that tumors with a high FAL value show a better response to a cisplatin-based chemotherapy than tumors with a low FAL value. To discover a potential clinical use of the FAL score for the prediction of response, we calculated the sensitivity, specificity, PPV, and NPV for a FAL value of >0.5. Although the specificity, PPV, and NPV were relatively high (93%, 82%, and 72%, respectively), the sensitivity (45%) was far too low to be useful for clinical practice. Chemotherapy response is considered to be highly complex, depending on tumor-specific characteristics as well as on constitutional genetic factors of the individual patient. Thus, it is unlikely that only one specific parameter will be found that will precisely predict therapy response for all patients. By enlarging the study population and analyzing other parameters that may be important for cisplatin and 5-FU-containing therapy regimens (for example, thymidylate synthase or ERCC1 [4–6]), it should be possible to refine therapy prediction by defining a prediction score based on the combination of different parameters. Given

and is thought to reflect the stabilization of the p53 protein by most of the missense mutations (42). However, we also observed a considerable number of tumors (n = 9) that were positive for p53 overexpression but were without mutation. Similar findings have been reported for gastric cancer as well as other tumor types (28, 29). It has been hypothesized that this may reflect a nonmutational stabilization of the p53 protein as a result from viral or oncogene binding (29). In addition, an overexpression of p53 induced by specific stress signals in the cell cannot be excluded. Furthermore, although we have demonstrated previously that DHPLC is a highly sensitive method for the detection of p53 mutations (19), we cannot completely be sure that every mutation was found.

Overall, p53 mutations were found more frequently in tumors exhibiting LOH at chromosome 17p13 (60%) than in informative tumors that did not demonstrate LOH (24%). An association with response, however, was only seen with LOH, and not with p53 mutations. An inactivation of a tumor suppressor gene by somatic mutation and concomitant LOH is the classic mechanism for tumor suppressor gene deactivation, but our findings indicate that other mechanisms unrelated to p53 contribute to the association of LOH with therapy response. It is well known (27, 28) that the association between mutation and LOH is not complete which is also seen for gastric cancer, and our data support this, and it has been hypothesized that other tumor suppressor gene(s) besides the p53 gene may reside in this chromosomal region (44). If there are two or more tumor suppressor genes, LOH at this chromosomal region might be even more critical for gastric tumorigenesis. Thus, an association of LOH at chromosome 17p with therapy response may suggest that the underlying mechanism for LOH, e.g., chromosomal instability, is more causally related to therapy response than merely the inactivation of p53. Furthermore, in the study as a whole, p53 mutations were found more frequently in tumors demonstrating a high FAL value (64%) or a medium FAL value (64%) than in tumors with a low FAL value (14%). Others have reported the concurrence of p53 mutations with genetic instability [i.e., aneuploidy (12, 13)], which is in line with our findings. However, recent studies of isogenic colorectal cell lines have demonstrated that there is no causal relationship between the concurrence of p53 mutation and chromosomal instability (45). This, in addition, could explain the lack of a clear association of p53 mutation with response in our study, and it suggests that factors besides p53 are important for the maintenance of genetic stability. Given the numerous genes that are involved in the correct chromosome segregation and maintenance of chromosomal stability, the global characterization of chromosomal instability may be more important for response prediction than the specific characterization of a single genetic factor.

Fig. 3 Comparison of the FAL value (percentage) of the tumors in relation to therapy response and patient survival. A, comparison of the FAL value (percentage) of the tumors between responders and nonresponders. B, Kaplan-Meier survival curves according to FAL.
the good predictive value of FAL for response to cisplatin-containing combinations, this value may be part of a set of useful prediction parameters, thus contributing to the selection of appropriate regimens for individual patients in the future.

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