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Allelic Imbalance at *p53* and Microsatellite Instability Are Predictive Markers for Resistance to Chemotherapy in Gastric Carcinoma

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Abstract

Background—Combined treatment with 5-fluorouracil and cisplatin (FP chemotherapy) is an effective neoadjuvant regimen for gastric carcinoma. However, it is ineffective in half of all patients. This study tests the hypothesis that genetic markers might identify those patients with gastric cancer who would respond to neoadjuvant FP chemotherapy.

Materials and Methods—A total of 23 patients with gastric carcinoma were treated with neoadjuvant chemotherapy. Pretreatment biopsy specimens before neoadjuvant chemotherapy were obtained from 15 of 23 patients, and resected tumors were obtained from all 23. Genetic studies were performed to detect allelic imbalance (AI), microsatellite instability (MSI), and *K-ras* mutation.

Results—A clinical response was observed in 13 of 23 patients. Kaplan–Meier survival curve showed that clinical responder group had a significantly higher likelihood of overall survival ($P = 0.0165$), compared with nonresponder group. In 23 resection specimens, 10 of 23 tumors presented AI at the *p53* locus and/or MSI; 8 of the 10 tumors were nonresponders, while 12 of 13 tumors without *p53* AI or MSI were responders ($P = 0.0007$). In 15 pretreatment biopsy specimens, 8 tumors had *p53* AI and/or MSI; 7 of the 8 tumors were nonresponders, while 6 of 7 tumors without *p53* AI or MSI were responders to preoperative chemotherapy ($P = 0.008$). Tumors with AI at the *p53* locus and/or MSI were significantly more resistant to neoadjuvant chemotherapy. No relationship was found between *K-ras* mutations and responses.

Conclusions—Analysis for *p53* AI and MSI might represent a clinically useful approach to predicting the response to neoadjuvant FP chemotherapy in gastric carcinoma.

Chemotherapeutic regimens are important for patients with recurrent or inoperable disease and are used as adjuvant therapy after surgery. Neoadjuvant chemotherapy increased the resectability of tumors and reduced the risk of postoperative recurrences, resulting in superior long-term survival.^{1–3} The combination of continuous 5-fluorouracil (5-FU) and low-dose cisplatin infusion (FP chemotherapy) has synergistic activity and is recognized as an effective

regimen for gastric carcinoma.⁴ Recently, data from several randomized trials reported that neoadjuvant chemotherapy with cisplatin and 5FU has a proven survival benefit in gastric cancer.^{5–7} Neoadjuvant FP chemotherapy for gastric cancer has a reported response rate of ~50%, exceeding that of other drug combinations.⁴ Contrariwise, FP chemotherapy is ineffective in half of all patients, and many will experience toxicity. The selection of patients with chemosensitive tumors before initiating neoadjuvant chemotherapy would be important for avoiding potential therapy-related complications and inappropriate delay of surgical treatment. Although various in vivo and in vitro approaches have been attempted to predict the chemosensitivity of individual tumors, reliable parameters have not been identified.^{8,9}

Tumorigenesis proceeds through the progressive accumulation of genomic instability in tumor suppressor genes and oncogenes such as *K-ras*.^{10,11} The loss of wild type tumor suppressor genes through allelic imbalance (AI) or “loss of heterozygosity” has been identified consistently in gastric cancer, particularly for the *p53* gene.¹² The mutated *K-ras* transfection was reported to decrease the ability of the cell to undergo apoptosis in response to thymidine deprivation.¹³ Because TS inhibition and subsequent thymidine deprivation is one of the main mechanisms of 5-FU cytotoxicity, the above findings suggest that *K-ras* mutation may influence 5-FU efficacy. Another genetic pathway for tumor progression is through the accumulation of DNA replication errors (called microsatellite instability, or MSI), which are a consequence of defective DNA mismatch repair (MMR) activity.¹⁴ MSI is the form of genomic instability that provides the mutational load in 10–20% of all sporadic gastric cancers.¹¹ Chromosomal instability and MSI are independent processes in cancer, although there is some degree of overlap in gastrointestinal cancers.^{15,16}

Several studies have linked inactivation of both the *p53* gene and MMR system in gastrointestinal carcinomas to reduced chemosensitivity in vitro.^{17,18} Recently, several clinical confirmations in this area are available.^{8,19,20} This study tests the hypothesis that one might be able to predict the chemosensitivity of neoadjuvant chemotherapy for gastric carcinomas by assessing genetic alterations.

MATERIALS AND METHODS

Patients

A total of 23 patients with gastric cancer that was considered to be stage III or locally advanced inoperable disease, as evaluated by computed tomography or ultrasonography, were treated with an FP regimen before surgery as neoadjuvant chemotherapy. No patient had received radiotherapy or other type of chemotherapy before FP. Patients ranged in age from 45 to 79 years (mean, 62.5). The General Rules for Gastric Cancer were used for pathological diagnosis and classification of variables.²¹ Eligibility for the study required an age not exceeding 80 years and an Eastern Cooperative Oncology Group performance status of 0 or 1. Informed consent was obtained from all patients prior to entry. The FP chemotherapy treatment schedule called for continuous 5-FU (350 mg/m²/day by continuous infusion) and low-dose cisplatin (7.5 mg/m²/day in 100 ml of normal saline infused over 1 h on days 1–5 of each week). This treatment was repeated weekly for 4 consecutive weeks, followed by a 4-week rest period. The age, gender, tumor location, lymphatic invasion status, and pathologic tumor lymph node metastases (pTNM) stage were evaluated by reviewing the medical charts and pathology records.²² Glass slides were reviewed to determine the histologic type according to the Lauren classification systems.²³ The clinical outcome of the patients was followed from the date of surgery to either the date of death or December 1, 2007, resulting in a follow-up that ranged from 1 to 120 months. This study was approved by the Osaka City University ethics committee. Informed consent was obtained from all patients prior to entry.

Assessment of Response

Patients were evaluated for response every 4 weeks with standard chest radiographs, computed tomography, ultrasonography, or upper gastrointestinal radiography. Objective responses quantified by image analysis were classified according to WHO criteria. A “response” was defined as a 50% or greater reduction of the longest perpendicular diameters of the size of the primary tumor as measured by computed tomography scan, endoluminal ultrasound, and total flattening of the gastric wall in endoscopy, provided that no new lesions appeared for at least 4 weeks. A “nonresponding” was defined as a reduction of less than 50%, or progression. Histological assessment proceeded according to The General Rules for Gastric Cancer.²¹ Grade 0 denoted no change with no necrosis or cellular or structural change visible throughout the lesion. Grade 1 denoted a slight change with Grade 1a or 1b, respectively, assigned when necrosis or disappearance of tumor involved less than 1/3 or no more than 2/3 of the lesion. Grade 2 denoted a moderate change with necrosis or disappearance of tumor involving more than 2/3 of the lesion. Two investigators independently determined the histological assessment of FP chemotherapy. When the determinations of the two investigators differed, assessment of response was judged through discussion.

DNA Extraction

All 23 patients underwent gastrectomy after FP chemotherapy. Pretreatment biopsy specimens were obtained from 15 of 23 patients during endoscopic examination at our institution prior to FP neoadjuvant chemotherapy. Biopsy specimens from the other 8 patients were not available because they were obtained at other hospitals. In the 23 resected tumors and 15 available biopsy specimens, DNA was extracted from the tissue section after it had been fixed in 10% buffered formalin and embedded in paraffin. One section was stained with hematoxylin and eosin and used as a reference for selecting areas for microdissection from adjacent sections, using a sterile scalpel blade under a dissecting microscope. Genomic DNA was isolated from the paraffin-embedded microdomains removed from the sections using Proteinase K (Gibco Life Technologies, Gaithersburg, MD).

Polymerase Chain Reaction (PCR)—Allelic Imbalance Assay

Five microsatellite loci, all linked to known tumor suppressor genes, were used as genetic markers of genomic instability. DNA was amplified by PCR using 5' 32P-end-labeled primers for the microsatellite loci: *D5S107*, *D10S541*, *D16S752*, *p53MEL*, and *D18S34*. These are linked to the *APC/MCC* loci on 5q21, the *PTEN* locus on 10q, the *E-cadherin* locus on 16q, the *p53* locus on 17p13, and the *DCC/DPC4* locus on 18q21.3, respectively. *BAT25* and *BAT26* are mononucleotide repeats. A total of 34 cycles of 93°C for 1 min, 55°C for 2 min, and 72°C for 2 min were performed with an initial denaturation step at 94°C for 2 min and a final extension step of 72°C for 10 min. PCR was repeated more than twice to ensure that the results were reproducible in each case. Allelic imbalance (AI) was considered to have occurred when an allele showed at least a 50% reduction in relative intensity in the neoplastic tissue compared with matched normal DNA.

Identification of Tumors with MSI

We used stringent criteria for the determination of MSI as specified by a National Cancer Institute Workshop.²⁴ A novel band shift was considered to be present when more than 20% of autoradiograms with informative microsatellite markers exhibited one or more extra alleles in the tumor sample. MSI-high (MSI-H) was defined as the detection of a novel band shift or allele at 30% or more of microsatellite loci tested when compared with nonneoplastic tissue from the same patient. MSI-low (MSI-L) was defined as the detection of a novel band shift or allele in fewer than 30% but more than 0% of the microsatellite loci tested. Microsatellite stable (MSS) was defined as the absence of novel band shifts in any marker. All assays for MSI were

based on PCR amplification using the panel of 7 microsatellite primer pairs as described. Samples positive for MSI were not included as informative at that locus in the AI analyses.

K-ras Mutation Analysis

DNA was amplified by hemi-nested PCR. Amplification of exon 1 of a *K-RAS* fragment containing codons 12 and 13 was performed using the following primers: forward, 5'-CGTCCACAAAATGATTCTGAATTAGCTGTATC-3'; and reverse, 5'-CCTTATGTGTGACATGTTCTAATATAGTCAC-3'. A total of 35 cycles (92°C for 30 s and 67°C for 30 s) were carried out, followed by 10 min of final extension at 72°C. These PCR products were diluted for a second round of amplification with the new forward primer 5'-AGGCCTGCTGAAAATGAC-3' and the same reverse primer used for the first round of PCR. A total of 35 cycles (92°C for 25 s, 55°C for 25 s and 72°C for 25 s) were carried out followed by 10 min of final extension at 72°C. The 104-bp amplicons were then dot-blotted onto nylon filters (Hybond-N; Amersham, Buckinghamshire, UK) and hybridized with radiolabeled oligomer primers representing all possible mutations at codon 12 and the GAC mutation at codon 13, as previously reported.²⁵

Statistics

Sensitivity to preoperative chemotherapy was investigated with reference to AI at the 5 loci, and to MSI. The Fisher exact test or Mann–Whitney *U* test was used for statistical analysis. *P* values <0.05 were considered statistically significant.

RESULTS

Relationships Between the Response to FP Chemotherapy and Clinicopathologic Factors

A responder was considered to indicate chemotherapy sensitivity and was observed in 13 of 23 cases (57%). Nonresponder, which defined chemotherapy resistance, was noted in 10 (43%). Histological assessment Grade 1b and Grade 2 was considered to indicate a histologic responder and was observed in 15 of 23 cases (65%). Histologic nonresponder as Grade 0 and Grade 1a was noted in 8 (35%). No significant relationship between response to FP chemotherapy and clinicopathologic factors was found (Supplemental Table A). Patients with no lymph node metastasis and a differentiation showed higher response rates than patients with lymph node metastasis and an intestinal-type, although this difference did not reach significance. Kaplan–Meier survival curves shows that the clinical responder group had a significantly higher likelihood of overall survival ($P = 0.0165$), compared with the nonresponder group (Fig. 1a). Taken together, the pathologic responder group (Grade 1b and Grade 2) had a significantly better survival ($P = 0.0487$), compared with the nonresponder group (Grade 0 and Grade 1a) (Fig. 1b).

Relationships Between Genetic Alterations in Biopsy Specimens and Clinical Chemosensitivity

Data represents 23 patients who were operated on at our institution and whose surgical specimens were available for examination. Pathologic response was correlated with patient outcome, with statistical significance; patients with response had better survival ($P = 0.0487$), compared with those with nonresponse.

Figure 2 demonstrates typical examples of AI (Fig. 2a) and MSI (Fig. 2b) at each locus. Informativity and AI results of biopsy specimens and resection specimens for all genes studied are presented in Table 1. Table 2 summarizes the relationships between chemosensitivity and genetic alterations in each of the biopsy specimens we obtained from 15 patients before neoadjuvant chemotherapy. AI of chromosome 17p was found in 5 of 15 informative biopsy

specimens (33%). All 5 informative biopsy specimens with AI at the *p53* locus were associated with resistance to preoperative chemotherapy, and a significant association was found between AI at the *p53* locus in the pretreatment biopsies and clinical chemoresistance ($P = 0.019$, Fisher exact test). AI of chromosomes 5q, 10q, 16p, or 18q was found in 2 of 10 (20%), 1 of 3 (33%), 1 of 9 (11%), and 2 of 14 (14%) informative biopsy specimens, respectively. No association was observed between chemosensitivity and AI at the *APC*, *DPC4*, *E-cadherin*, or *PTEN* loci was found. MSI-H was found in 3 of 15 informative biopsy specimens (20%). No statistically significant difference in chemosensitivity was demonstrated between MSI-H and MSI-L or MSS cases. The region encompassing codons 12 and 13 of the *K-RAS* gene was successfully amplified from all microdissected tissue foci. Of 15 informative biopsy specimens, 1 (7%) had mutations of *K-RAS* codon 12. No significant relationship was found between *K-RAS* mutations and response to FP therapy.

Of 8 tumors showing AI at the *p53* locus and/or MSI-H in the biopsy specimen, 7 (88%) were resistant to preoperative chemotherapy. In contrast, 6 of 7 tumors without AI at the *p53* locus or MSI-H in the biopsy specimen were sensitive to chemotherapy (86%; $P = 0.008$, Fisher exact test).

Relationship Between Genetic Alterations in 23 Resection Specimens and Clinical Chemosensitivity

Table 3 summarizes the relationship between chemosensitivity and genetic alterations in 23 gastric tumors as determined in the gastrectomy specimens obtained after chemotherapy. AI of chromosome 17p was found in 7 of 21 informative resected tumors (33%). Of 7 informative resected tumors with AI at the *p53* locus, 6 (86%) were resistant to FP chemotherapy ($P = 0.024$, Fisher exact test). In contrast, AI of chromosome 5q, 10q, 16p, and 18q was found in 5 of 15 (33%), 3 of 6 (50%), 3 of 11 (27%), and 5 of 17 (29%) informative resected specimens, respectively. No association was observed between chemosensitivity and AI at the *APC*, *PTEN*, *DPC4*, or the *E-cadherin* loci. MSI-H was found in 4 of 23 informative resected tumors (17%). No significant difference in the prevalence of MSI-H was found between responder and non-responder cases. Of 23 informative resected tumors, 3 (13%) had mutations of *K-RAS* codon 12. All 3 informative resected tumors with mutations of *K-RAS* were sensitive to FP chemotherapy, although a statistically significant relationship was not found between *K-RAS* mutations and response to FP therapy. In contrast, 8 of 9 tumors with AI at the *p53* locus and/or MSI-H (89%) were resistant to preoperative chemotherapy, while 12 of 14 tumors without AI at *p53* locus or MSI-H in the resected specimen were sensitive to chemotherapy (86%; $P = 0.0007$, Fisher exact test). Tumors with AI at the *p53* locus and/or MSI-H thus were significantly more resistant to preoperative chemotherapy than those without AI at the *p53* locus and MSI-H, based on either biopsy or resection specimens.

Relationship Between Genetic Alterations and the Histological Response Grade

Associations between the histological response grade and *p53*AI and/or MSI-H are summarized in Table 4. All tumors representing a Grade 0 response had AI at the *p53* locus and/or MSI-H in either the biopsy or and resection specimens. In contrast, all tumors with responses representing histological Grade 1b or 2 had neither AI at the *p53* locus nor MSI-H in the biopsy or resection specimens. AI at the *p53* locus and/or MSI-H were significantly associated with histological response grade in both biopsy specimens ($P = 0.001$, Mann–Whitney *U* test) and resected tumors ($P = 0.0003$, Mann–Whitney *U* test). Cases 7 and 17 showed a change in the results for *p53* AI between the prechemotherapy and postchemotherapy samples. Both cases had histological response Grade 1a and showed *p53* AI in the resection specimens, but not in the pretreatment biopsy samples.

DISCUSSION

A randomized phase III trial of chemotherapy in patients with resectable gastric cancer demonstrated a survival benefit with the use of perioperative chemotherapy compared with surgery alone, with an estimated improvement of 13 percentage points in the 5-year survival rate, corresponding to a 25% reduction in the risk of death.⁵ In this study, Kaplan–Meier survival curves indicated the significant correlation of response and survival of the patients. These findings suggested that neoadjuvant chemotherapy with cisplatin and 5FU has proven benefit in patients with gastric cancer. In this study, the overall response rate to neoadjuvant FP chemotherapy was 57%, a result superior to that reported previously for other neoadjuvant chemotherapies. However, 10 of 23 patients had an inadequate clinical response to FP chemotherapy, and it could be argued that they were treated unnecessarily. The toxicities of FP chemotherapy were not life threatening; nonetheless, leucopenia, thrombocytopenia, nausea, and vomiting occur commonly with this regimen.⁴ The purpose of neoadjuvant chemotherapy is to decrease the aggressiveness of the tumor prior to surgery, to control dissemination of cancer cells during the operation, and to limit the growth of micrometastases at an early stage. Although neoadjuvant chemotherapy has been reported to improve outcomes, ideally, sensitive tumors should be identified before chemotherapy to avoid delaying surgery and to prevent unnecessary drug toxicity.^{1–3} A relationship between the response to FP therapy and clinicopathologic factors had not been apparent previously.^{4,26} In our study, FP chemosensitivity was not reliably predicted by clinicopathologic features, which directed our focus to the potential use of genetic alterations as predictors of chemosensitivity.

The objective response degree was initially divided into four groups as a complete response (CR) as defined as the disappearance of all measurable lesions, a partial response (PR) as defined as a reduction of at least 50%, stable disease (SD) as defined as a reduction of less than 50% or an increase of less than 25%, and progressive disease (PD) as defined as an increase of greater than 25% in tumor size or the appearance of new lesions. However, CR or PD was not observed in any cases in this study. Then, objective response was classified as 13 cases of responder as PR and 10 cases of nonresponder as SD. Effect of neoadjuvant chemotherapy was followed for only 8 weeks. This observation period of 8 weeks might be too short to evaluate for CR or PD.

In resected specimens, 7 of 21 (33%) of the gastric cancers had AI at 17p. Of 7 informative resected tumors with AI at the *p53* locus, 6 (86%) were resistant to FP chemotherapy, suggesting a significant association between *p53* gene deletion and chemoresistance, whereas no significant relationship was found between the frequency of AI at 5q, 10q, 16p, or 18q and chemoresistance. Thus, AI at 17p was important for predicting chemosensitivity. MSI-H was found in 3 of 10 (30%) of resected tumors with nonresponder, and in 1 of 13 (8%) of informative tumors with responder. MSI-H tumors therefore tended to be chemoresistant, although statistical significance was not reached in this small study. In the biopsy specimens, a strong influence of 17p AI and/or MSI-H on chemoresistance was also recognized. Grundei et al. have reported that AI at 17p13 was associated with a better clinical response to cisplatin-based chemotherapy, suggesting that altered *p53* function might render cells more sensitive to chemotherapy, which is contradictory to our findings.²⁰ It is not clear why there are such discrepant findings between that study, from Germany, and our results obtained on Japanese patients, but the data we obtained were highly significant, even in this relatively small study. Controversy remains regarding whether the loss of *p53* might render cells relatively resistant to chemotherapy, but few suggest that loss of *p53* makes cells more sensitive to cytotoxic insults. Our data are more consistent with the mainstream observations and interpretations.^{27–29} Thymidylate synthase (TS) regulates the production of DNA synthesis precursors and is an important target of cancer chemotherapy. A polymorphic tandem repeat sequence in the enhancer region of the TS promoter was described, where the triple repeat gives higher in vitro

gene expression than a double repeat. Recently ethnic differences in TS polymorphisms were identified between Caucasian and Asian populations.³⁰ The differences of TS polymorphisms among the Asian and European patients might influence the different response to 5-FU.

All of our cases with a Grade 0 pathological response showed nonresponder, and all cases with Grade 2 responses had responders, which indicated a significant relationship between the histological response grade and the clinical response. All histological Grade 0 responses came from cases with AI at the *p53* locus and/or MSI-H. In contrast, all cases with Grade 1b or 2 histological responses showed neither AI at the *p53* locus nor MSI-H. The above findings strongly suggested that *p53* AI and MSI would be clinically useful as predictive markers for neoadjuvant FP chemotherapy, although a larger study will be necessary to confirm the efficacy.

K-RAS mutations were infrequent among gastric carcinomas (13% of resected tumors), as previously reported.³¹ Although no significant relationship between *K-RAS* mutations and response to FP chemotherapy was found, all three gastric cancers with *K-RAS* mutations showed a good response to chemotherapy. This finding suggested that tumors associated with the increased cell growth activated by *K-RAS* mutations might still be sensitive to chemotherapy.

Cisplatin therapy not only modulates the pharmacologic effect of 5-FU, but also exerts a direct antitumor effect on cancer cells.⁴ The cytotoxicity of cisplatin is mediated by the formation of interstrand DNA cross-links, while 5-FU has multiple effects, among which is incorporation into both RNA and DNA.^{17,32} The p53 protein is recognized as an important regulatory element that signals growth arrest in cells containing damaged DNA. Activation of apoptosis in cancer cells containing damaged DNA induced by chemotherapy is closely linked to p53 activity.^{33, 34}

MMR system consists of a complex of proteins that recognizes and directs repair of nucleotide base mismatches and of slippage at simple repetitive sequences termed microsatellites.¹⁴ Detection of damaged DNA by an intact MMR system signals events that may lead to apoptotic cell death.³⁵ The human MMR system can also recognize DNA adducts consisting of interstrand cross-links formed by cisplatin and 5-FU, while loss of DNA MMR activity directly contributes to cisplatin resistance in vitro.^{17,36} Our present findings suggest that *p53* loss and inactivation of the MMR system may promote the survival of cancer cells with damaged DNA after FP chemotherapy. Thus, *p53* AI and MSI-H have clinical promise as markers for chemoresistance to combined 5-FU and cisplatin therapy for gastric cancer.

There are relatively few studies that investigate the mechanisms involved in chemosensitivity using specimens obtained both before and after chemotherapy. Two of our 15 patients showed a difference in *p53* AI status between the preoperative biopsy and the resection specimens. In both instances, the biopsy specimens were *p53* AI negative, but the resected specimens were *p53* AI positive. One explanation for this apparent change might involve preexisting heterogeneity of *p53* AI in the tumor, allowing cancer cells with wild-type *p53* to be killed by the chemotherapy, leaving *p53* AI-positive, chemoresistant cancer cells. In contrast, no difference in MSI-H status was found between biopsy specimens and resected samples in 15 patients. This suggests that tumors with MSI-H are more likely to be homogeneous for this form of genomic instability and consistent with the concept that MSI represents a unique pathway of tumor development and that DNA MMR deficient cells are not often admixed with those competent in MMR prior to therapy.

About 50% of gastric cancer patients have tumors with either *p53* AI (40% of tumors) or a defective MMR system (10% are MSI-H). These patients might not benefit from neoadjuvant chemotherapy. We propose the following strategy for the treatment of patients with advanced

gastric cancer. A genetic evaluation of a biopsy specimen would be used to assist the decision of whether or not neoadjuvant chemotherapy should be given. If the biopsy specimen from a gastric cancer shows no evidence for *p53* AI and is MSS, the patient would be more likely to benefit from treatment. If the initial FP chemotherapy is effective, biopsy and genetic analyses should precede the decision for a second course of FP chemotherapy. If a biopsy specimen obtained after initial FP chemotherapy still shows *p53* AI negativity and MSS, the patient might benefit from a second course of FP. This strategy should be tested in a prospective, controlled clinical trial.

In conclusion, cancer cells resistant to chemotherapy may escape apoptosis by loss of the *p53* gene or loss of the MMR system. These two mechanisms reflect the respective results of chromosomal instability and MSI, respectively. Analysis of tumor DNA for *p53* AI and MSI might represent a clinically useful approach to predicting the response to neoadjuvant FP chemotherapy in gastric carcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Kaplan–Meier estimates of overall survival. **a** Clinical response and survival. Compared with the nonresponder group, the responder group had a significantly higher likelihood of overall survival ($P = 0.0165$). **b** Pathological response and survival. Data represents 23 patients who were operated on at our institution and whose surgical specimens were available for examination. Pathologic response was correlated with patient outcome, with statistical significance; patients with response had better survival ($P = 0.0487$), compared with those with nonresponse. Comparison of *p53* allelic imbalance (c) and *p53* AI/MSI (d), in relation to survival. Although there was no significant survival difference ($P = 0.0764$) between patients with or without *p53* allelic imbalance (AI), significant survival difference ($P = 0.0133$) was shown between patients with or without *p53* AI/MSI.

FIG. 2. Allelic imbalance (a) and microsatellite instability (b) at chromosomes 2p, 3p, 5q, 17p, 18q, BAT25, and BAT26 microsatellite markers in DNA from a pretreatment biopsy specimen (Lane B), the resection specimen (Lane T), and corresponding normal tissue (Lane N). Electrophoresis was carried out in 8% polyacrylamide gels. Arrowheads show allelic imbalance (AI) and band shifts

TABLE 1

Genetic alterations for each individual cancer

Case	Response assessment		Specimen	Chromosomal locus (<i>gene</i>)					BAT25	BAT26	MSI ^a	K-ras ^b
	Clinical	Histology		17p (<i>p53</i>)	5q (<i>APC</i>)	10q (<i>PTEN</i>)	16p (<i>E-Cadherin</i>)	18q (<i>DPC4</i>)				
1	Nonresponder	0	Biopsy	AI	None	Hom	AI	-	-	-	0/6	-
			Resection	AI	AI	Hom	AI	-	-	-	0/7	-
2	Nonresponder	0	Biopsy	AI	None	Hom	Hom	AI	-	-	0/6	-
			Resection	AI	-	Hom	Hom	AI	-	-	0/7	-
3	Nonresponder	0	Biopsy	AI	Shift	None	Shift	Shift	-	Shift	4/6	MSI-H
			Resection	AI	Shift	None	Shift	Shift	-	Shift	4/6	MSI-H
4	Nonresponder	0	Biopsy	AI	-	None	Hom	-	-	-	0/6	-
			Resection	AI	-	None	Hom	-	-	-	0/6	-
5	Nonresponder	0	Biopsy	AI	AI	Hom	-	Hom	-	-	0/7	-
			Resection	AI	AI	None	-	Hom	-	-	0/6	-
6	Nonresponder	0	Biopsy	Shift	None	Shift	Hom	-	Shift	Shift	4/6	MSI-H
			Resection	Shift	None	Shift	Hom	-	Shift	Shift	4/6	MSI-H
7	Nonresponder	1a	Biopsy	-	None	Hom	-	-	-	-	0/6	-
			Resection	AI	AI	Hom	-	-	-	-	0/7	-
8	Nonresponder	0	Resection	Shift	-	Shift	-	-	Shift	-	3/7	MSI-H
9	Nonresponder	0	Biopsy	-	Shift	Hom	Hom	-	-	-	1/7	MSI-L
			Resection	-	Shift	None	Hom	-	-	-	1/6	MSI-L
10	Nonresponder	1b	Resection	-	-	Hom	Hom	-	-	-	0/7	-
11	Responder	2	Biopsy	-	-	Hom	-	-	-	-	0/7	-
			Resection	-	-	Hom	-	-	-	-	0/7	-
12	Responder	1b	Biopsy	-	AI	-	-	-	-	-	0/7	-
			Resection	-	AI	-	AI	AI	-	-	0/7	-
13	Responder	1a	Biopsy	-	-	Hom	Hom	-	-	-	0/7	12Asp
			Resection	-	-	Hom	Hom	-	-	-	0/7	12Asp
14	Responder	1b	Biopsy	-	None	Hom	Hom	-	-	-	0/6	-
			Resection	-	-	Hom	Hom	-	-	-	0/7	-
5	Responder	1a	Biopsy	-	-	Hom	-	AI	-	-	0/7	-

Case	Response assessment		Specimen	Chromosomal locus (<i>gene</i>)				BAT26	BAT25	MSI ^a	K-ras ^b	
	Clinical	Histology		17p (<i>p53</i>)	5q (<i>APC</i>)	10q (<i>PTEN</i>)	16p (<i>E-Cadherin</i>)	18q (<i>DPC4</i>)			Number of markers mutated	Status
16	Responder	2	Resection	–	–	Hom	–	AI	–	–	0/7	–
			Biopsy	–	–	None	–	–	–	–	0/6	–
			Resection	–	–	None	AI	–	–	–	0/6	–
17	Responder	1a	Biopsy	–	Shift	AI	–	Shift	–	–	3/7	MSI-H
			Resection	AI	Shift	AI	–	Shift	–	–	3/7	MSI-H
18	Responder	1a	Resection	–	–	Hom	–	–	–	–	0/7	–
19	Responder	1a	Resection	–	Shift	–	Shift	–	–	–	2/7	MSI-L
20	Responder	1b	Resection	–	None	None	–	–	–	–	0/5	–
21	Responder	2	Resection	–	–	None	–	AI	–	–	0/6	12Asp
22	Responder	1a	Resection	N.I.	–	None	–	–	–	–	0/5	–
23	Responder	1b	Resection	Hom	AI	AI	Hom	AI	–	–	0/7	12Cys

Histology refers to change in tumor grade in response to chemotherapy (see Materials and Methods)

None no amplification at PCR and no band obtained, *AI* allelic imbalance, *MSI-L* microsatellite instability-low, *MSI-H* microsatellite instability-high, *Shift* band shift indicative of MSI, *Hom* homozygous (not informative), – no AI, MSS, or no mutation

^aMSI-high (MSI-H) was defined as a presence of a novel allele at $\geq 30\%$ of microsatellite loci tested compared with non-neoplastic tissue from the same patient, and MSI-low (MSI-L) as a novel allele at $< 30\%$ but $> 0\%$ of microsatellite loci tested. Microsatellite stable (MSS) was defined when none of the markers exhibited a novel allele

^bMutations are shown with the number of the codon involved and the new amino acid encoded: *Asp* codon 12 aspartic acid, *Cys* codon 12 cysteine

TABLE 2

Correlation between chemosensitivity and the genetic alterations in 15 biopsy specimens obtained before neoadjuvant FP chemotherapy

Genetic alterations	Clinical assessment ^a		P value*
	Nonresponder	Responder	
Allelic imbalance (AI)			
<i>p53</i> (17p)			
AI (+)	5	0	0.019
AI (−)	3	7	
<i>APC</i> (5q)			
AI (+)	1	1	NS
AI (−)	3	5	
<i>DPC4</i> (18q)			
AI (+)	1	1	NS
AI (−)	6	6	
<i>E-Cadherin</i> (16p)			
AI (+)	1	0	NS
AI (−)	3	5	
<i>PTEN</i> (10q)			
AI (+)	0	1	NS
AI (−)	1	1	
MSI-H			
(+)	2	1	NS
(−)	6	6	
<i>K-ras</i> mutation			
(+)	0	1	NS
(−)	8	6	
<i>p53</i> AI and MSI-H			
Either positive	7	1	0.008
Both negative	1	6	

AI allelic imbalance, MSI-H microsatellite instability-high, NS not significant

* Significance level of difference was determined using Fisher exact test

^a Response assessment was defined as described in the Materials and Methods

TABLE 3

Correlation between chemosensitivity and genetic alterations in 23 operative specimens

Genetic alterations	Clinical assessment ^a		P value [*]
	Nonresponder	Responder	
Allelic imbalance			
<i>p53</i> (17p)			
AI (+)	6	1	0.024
AI (−)	4	10	
<i>APC</i> (5q)			
AI (+)	3	2	NS
AI (−)	4	6	
<i>DPC4</i> (18q)			
AI (+)	1	4	NS
AI (−)	7	5	
<i>E-Cadherin</i> (16p)			
AI (+)	1	2	NS
AI (−)	2	6	
<i>PTEN</i> (10q)			
AI (+)	2	1	NS
AI (−)	2	1	
MSI-H			
(+)	3	1	NS
(−)	7	12	
<i>K-ras</i> mutation			
(+)	0	3	NS
(−)	9	10	
<i>p53</i> AI/MSI-H			
Either positive	8	1	0.0007
Both negative	2	12	

NS not significant, AI allelic imbalance, MSI-H microsatellite instability-high

^{*} Significance of the difference was determined using Fisher exact test^a Response assessment was defined in the Materials and Methods

TABLE 4

Correlation between *p53* AI and/or MSI and histological response to therapy

Specimen	<i>p53</i> AI/MSI	Histologic assessment ^d		<i>P</i> value [*]
		Grade 0	Grade 1a	Grade 1b or 2
Biopsy specimen (<i>n</i> = 15)	Either positive	7	1	0
	Both negative	0	3	4
Operative specimen (<i>n</i> = 23)	Either positive	8	2	0
	Both negative	0	5	8

^{*} Significance level of difference was determined using Mann–Whitney *U* test

^d Response assessment is the change in tumor histology after chemotherapy, as defined in the Materials and Methods