TP53 and Long-Term Prognosis in Colorectal Cancer: Mutations in the L3 Zinc-binding Domain Predict Poor Survival[1]

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ABSTRACT

In a consecutive series of 222 colorectal carcinomas from patients with a median follow-up time of 56.8 months (range, 0.5–92.2) treated with surgery, the *TP53* gene was screened for mutations. Exons 5–8 were analyzed using constant denaturant gel electrophoresis followed by sequencing, and mutations were found in 102 cases (45.9%). Mutations were found more frequently in rectal tumors *versus* other locations ($P = 0.029$) and in aneuploid compared to diploid tumors ($P < 0.001$). Presence of a *TP53* mutation was also significantly associated with absence of microsatellite instability ($P = 0.028$), as well as with loss of heterozygosity at 17p13 ($P < 0.001$). The *TP53* mutations in the left-sided and rectal tumors were more often transversions than transitions, indicating a different etiology in the development of these tumors. The tendency for shorter cancer-related survival among patients with mutations in their tumors was only statistically significant for patients with left-sided tumors ($P = 0.003$). All patients with mutations affecting the L3 domain of the protein involved in zinc binding had a significantly shorter cancer-related survival ($P = 0.036$), indicating that mutations affecting this domain have biological relevance in terms of colorectal cancer disease course. These results suggest that knowledge of a patient's *TP53* status, with respect to both the presence and the localization of the mutation, may be important in prognosis evaluation, particularly when selecting patients for more aggressive postoperative therapeutic intervention.

INTRODUCTION

CRC[3] is the second most common cause of cancer mortality in the Western world. Clinical and pathological characteristics of CRC permit prediction of survival probability following surgery. The current staging methods used to assess the prognosis in patients with CRC give 90% specificity and 40% sensitivity (1, 2). Thus, a significant proportion of patients with early-stage disease develop recurrent disease. The mainstay treatment for CRC is surgical resection, which may be either curative or palliative, depending on the spread of the disease. Selected patients may be judged to be suitable for additional postoperative treatment in an attempt either to prevent growth of secondary cancers and/or to reduce the tumor burden. Genetic markers may identify subsets of such patients who will benefit from more aggressive therapy, and thereby may be usable in clinical evaluation.

Colorectal carcinomas arise as a result of activation of oncogenes and inactivation of tumor suppressor genes. Inactivation of the tumor suppressor gene *TP53* is an important step in the development of many human cancers, including CRC (3, 4). Mutations in the *TP53* gene occur late in tumor progression and are observed in 50–70% of human colorectal carcinomas (5, 6). Some studies have reported an association between the presence of *TP53* mutations and poor prognosis (6–9). Others have failed to observe such an association (10, 11), especially when immunohistochemistry has been used for mutation detection (12–17).

The *TP53* gene is located at chromosome band 17p13.1 and encodes a multifunctional transcription factor that orchestrates cellular responses following DNA damage, including G_1 and G_2 growth arrest and apoptosis (18–22). The p53 protein plays a direct role in DNA repair (23, 24) and also seems to be involved in angiogenesis, senescence, and differentiation (25, 26). The protein is highly conserved and consists of several domains with different functions: a transactivating NH_2 terminus, a core domain with specific DNA-binding activity, and a COOH terminus involved in tetramerization (27). The crystal structure of the protein's core domain in complex with DNA has shown that this domain is made of a scaffold of two antiparallel β-sheets supporting a bipartite DNA-binding surface. This surface comprises the two loops, L2 and L3, that are held together by a zinc atom and interact with the minor groove of DNA, and a loop-sheet-helix motif that interacts with the major groove (Refs. 28 and 29; Fig. 1*a*). Most *TP53* mutations in colorectal carcinomas are found in the part of the gene that encodes the core domain of the protein. The most frequent mutations are His175 in L2, Ser245 and Glu248 in L3, and His273 within the loop-sheet-helix motif

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[3] The abbreviations used are: CRC, colorectal cancer; CDGE, constant denaturant gel electrophoresis; LOH, loss of heterozygosity; MIN, microsatellite instability.

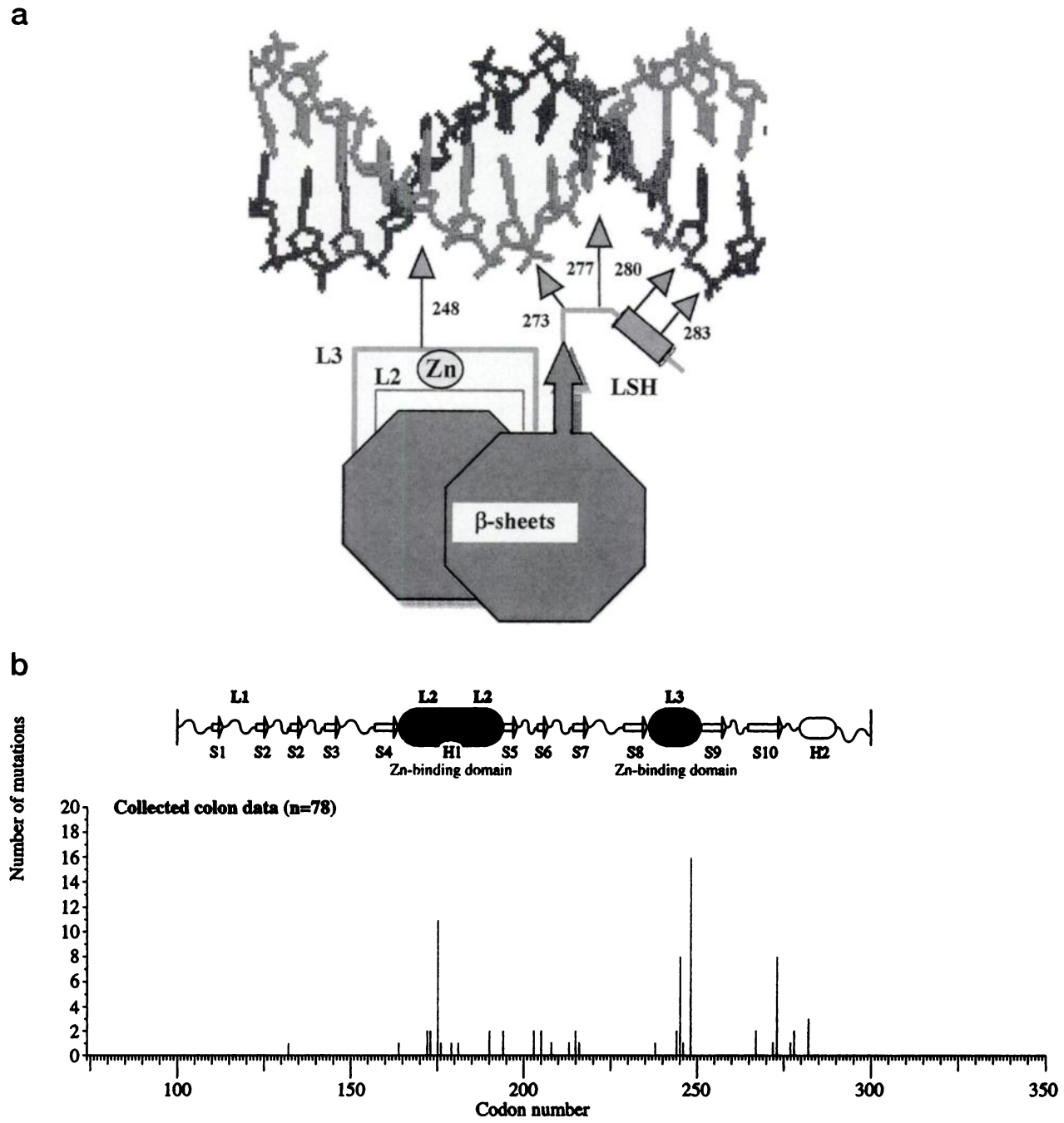

Fig. 1 *a*, schematic representation of the p53 protein core domain in complex with its target DNA. The special relationships between secondary structure elements are shown. The core domain is made of a scaffold of two β-sheets supporting the two loops, L2 and L3, which are held together by a zinc atom and bind in the minor groove of DNA. Another structural motif, loop-sheet-helix (*LSH*), binds in the major groove. Contacts in the minor groove involve essentially arginine 248, whereas several residues make DNA contacts in the major groove (including 273, 277, 280, and 283). Mutations of the amino acid arginine at 248 therefore disrupt totally the DNA binding to the minor groove, whereas mutations at position 273 or 283 only reduce the DNA binding to the major groove. The zinc atom keeps L3 in correct orientation and is thus crucial for interaction with the minor groove. *b*, distribution of the various *TP53* missense mutations in the core region of the gene detected in the present data set of colorectal carcinomas. *Top*, locations of the loops (*L*), sheets (*S*), and helices (*H*) in the protein compared to the gene structure. *Shaded areas*, amino acids involved in zinc binding.

(3, 4). Recent studies on both CRC and breast cancer patients have suggested that specific mutations are associated with more aggressive tumors, resulting in shorter survival and in tumors resistant to chemotherapy (7, 30–32).

To clarify the influence of different *TP53* mutations on long-term survival in patients with CRC, we performed mutation screening using CDGE followed by sequencing in a series of 222 primary tumors from patients who underwent surgery

Table 1 *TP53* status in relation to clinicopathological variables and other genetic changes in 222 colorectal cancer patients

	Number with mutations/ total number	Percentage	*P*
Sex			
Males	58/116	50%	
Females	44/106	41.5%	0.257
Age			
Males <60	16/25	64%	
Males ≥60	42/91	46%	0.175
Females <60	5/10	50%	
Females ≥60	39/96	41%	0.814
Tumor site			
Right colon	24/67	36%	
Left colon	21/52	40%	
Rectum	57/103	55%	0.029
Histological grade			
Well differentiated	4/14	29%	
Moderately differentiated	88/177	50%	
Poorly differentiated	10/31	32%	0.080
Dukes stage			
A	11/32	69%	
B	50/98	51%	
C	29/64	45%	
D	12/28	43%	0.414
Ploidy			
Diploid	19/86	22%	
Aneuploid	83/136	61%	<0.001
MIN status			
At two or more loci	3/17	18%	
At one or no loci	87/179	49%	0.028
LOH of 17p13			
Presence	73/121	60%	
Absence	9/59	15%	<0.001

alone as the curative treatment. The prior hypothesis was that mutations in the *TP53* gene, in particular mutations in the zinc-binding domain, and LOH at the *TP53* locus both reduce cancer-related survival.

MATERIALS AND METHODS

Patients. Tumor and blood samples from a consecutive series of 222 CRC patients, 116 males and 106 females, were collected from seven hospitals in the Oslo region during the period 1987–1989. The clinicopathological characteristics of the patients are given in Table 1. The mean age at diagnosis was 68.1 years (range, 26–94 years) for males and 68.9 years (range, 41–92 years) for females. This series included 67 tumors located in the right colon (cecum, ascendens, and right flexure), 52 tumors to the left colon (left flexure, descendens, and sigmoideum; this series did not contain tumors in the transversum), and 103 in the rectum. The median follow-up time of these patients was 56.8 months (range, 0.5–92.2 months) and was established as time between surgery and death or last update (January 1, 1996). All of the patients underwent surgery alone as the curative treatment except for a few patients with rectal tumors who also received postoperative radiation therapy. At the time of relapse, approximately one-third of the patients received palliative chemotherapy treatment. Two different end points were studied; cancer-related survival and overall survival. For the first end point, all events except CRC deaths were treated as censored. For the second end point, all events except deaths from any causes were treated as censored.

Materials. Cell suspensions from fresh tissue samples were mechanically prepared by mincing tumor samples in PBS followed by nylon mesh filtration (70 mm). The cells were fixed and stored in 70% ethanol at 4°C prior to DNA extraction. The fraction of normal cells in the tumor-cell suspensions has previously been estimated by cytological examination of cytospin preparations (33). From these studies, the cell suspensions were shown to contain from 62 to 97% tumor cells (mean, 84%). Blood and tumor samples were extracted with chloroform/phenol followed by ethanol precipitation using a 340ABI nucleic acid extractor. DNA was stored in 1× TE buffer [10 mM Tris (pH 8), 1 mM EDTA] at 4°C until analyses.

Genotype Analyses. LOH at two markers, pBHP53 and pYNZ22 (D17S30), located at chromosome bands 17p13.1 and 17p13.3 respectively, has previously been investigated in this series of tumors (34). The concordance between LOH of these two markers when both were informative was 97%, and when loss was demonstrated at pYNZ22, pBHP53 also always exhibited allele loss. Therefore, loss of either one or both of these markers was scored as LOH of 17p13 in the analyses performed in this study. MIN has previously been analyzed and was found in a subgroup (16%) of 241 analyzed CRCs, including 196 of the tumors studied here (35).

***TP53* Mutation Analyses.** Mutations in the *TP53* gene were analyzed by CDGE, as described elsewhere (36–38), with primers covering the evolutionary conserved regions of the gene, exons 5–8 (codons 126–300). This includes both the L2 and L3 zinc-binding domains covering codons 163–195 and codons 236–251, respectively. All samples with aberrant migrating bands on CDGE were submitted to direct sequencing of new PCR products using standard dideoxy sequencing reaction and Dynabeads M280-streptavidin (Dynal AS; Oslo, Norway) as solid support.

Statistical Analyses. Statistical analyses were conducted using Systat and SPSS software. Comparisons between different groups were performed using χ^2 analysis with Yates correction. Survival curves were estimated by the Kaplan-Meier method, and differences were assessed using the log rank test. The associations between *TP53* status and tumor location and LOH of 17p13 and the MIN status were of primary interest, as well as the associations between survival and status of the *TP53* gene, LOH of 17p13, and mutations in the zinc-binding domain. *P*s below 0.05 were regarded as statistically significant in these comparisons. Associations between mutations of the *TP53* gene and other clinicopathological variables were regarded as information of secondary interest. Thus, to account for multiple significance tests for these associations, only *P*s lower than 0.001 were regarded as statistically significant.

RESULTS

Among the 222 tumors analyzed, 102 cases (45.9%) revealed 105 *TP53* mutations within exons 5–8. The *TP53* status in relation to clinicopathological variables is shown in Table 1. A slightly higher frequency of mutations was found in males compared to females (50 *versus* 41.5%) and in the younger age groups. The proportion of *TP53* mutations seemed to be higher

Table 2 Characterization of the different *TP53* mutations found in the colorectal cancer patients described

Sample no.	Codon no.	Base change	Amino acid change
C 956	132	AAG to AGG	Lys to Arg
C 986	141	TGC to TGA	Cys to Stop
C 892	146	TGG to TAG	Trp to Stop
C 1265	164	AAG to GAG	Lys to Glu
C 1071	172	GTT to TTT	Val to Phe
C 1085	172	GTT to GAT	Val to Asp
C 1400	173	GTG to ATG	Val to Met
C 1038	173	GTG to TTG	Val to Leu
C 1025	175	CGC to CAC	Arg to His
C 1057	175	CGC to CAC	Arg to His
C 1098	175	CGC to CAC	Arg to His
C 1120	175	CGC to CAC	Arg to His
C 1155	175	CGC to CAC	Arg to His
C 1158	175	CGC to CAC	Arg to His
C 1196	175	CGC to CAC	Arg to His
C 1380	175	CGC to CAC	Arg to His
C 1403	175	CGC to CAC	Arg to His
C 1096	175	CGC to CAC	Arg to His
C 1382	175	CGC to CAC	Arg to His
C 1301	176	TGC to TAC	Cys to Tyr
C 1052	179	CAT to CTT	His to Leu
C 1030	181	CGC to CCC	Arg to Pro
C 1121	190	CCT to CTT	Pro to Leu
	267	CGG to TGG	Arg to Trp
C 927	190	CCT to CTT	Pro to Leu
C 1362	194	CTT to TTT	Leu to Phe
C 1046	194	CTT to CGT	Leu to Arg
C 932	196	CGA to TGA	Arg to Stop
C 1014	203	GTG to GAG	Val to Glu
C 1274	203	GTG to GAG	Val to Glu
C 1013	205	TAT to TGT	Tyr to Cys
C 1402	205	TAT to TGT	Tyr to Cys
C 971	208	GAC to GGC	Asp to Gly
C 1194	213	CGA to CTA	Arg to Leu
C 914	213	CGA to TGA	Arg to Stop
C 1031	213	CGA to TGA	Arg to Stop
C 1304	213	CGA to TGA	Arg to Stop
C 1334	213	CGA to TGA	Arg to Stop
C 1089	215	AGT to AAT	Ser to Asn
	212–216	TTCGACATAGTGTG to TTATGTG	7-bp deletion
C 1389	215	AGT to AAT	Ser to Asn
C 1026	216	GTG to TTG	Val to Leu
C 1095	238	TGT to CGT	Cys to Arg
C 1027	241–242	TCCTGC to TTCCGC	Ser, Cys to Phe, Arg
C 1091	244	GGC to GTC	Gly to Val
C 1296	244	GGC to GTC	Gly to Val
C 896	245	GGC to GAC	Gly to Asp
C 1037	245	GGC to AGC	Gly to Ser
C 1049	245	GGC to AGC	Gly to Ser
C 1164	245	GGC to AGC	Gly to Ser
C 858	245	GGC to AGC	Gly to Ser
	151–152	CCC CCG to CCCCG	1-bp deletion
C 1284	245	GGC to AGC	Gly to Ser
C 1357	245	GGC to AGC	Gly to Ser
C 903	245	GGC to AGC	Gly to Ser
C 1197	246	ATG to AGG	Met to Arg
C 1009	248	CGG to CAG	Arg to Gln
C 1067	248	CGG to CAG	Arg to Gln
C 1108	248	CGG to CAG	Arg to Gln
C 1115	248	CGG to CAG	Arg to Gln
C 1267	248	CGG to CAG	Arg to Gln
C 1272	248	CGG to CAG	Arg to Gln
C 1286	248	CGG to CAG	Arg to Gln
C 1287	248	CGG to CAG	Arg to Gln
C 1340	248	CGG to CAG	Arg to Gln
C 1368	248	CGG to CAG	Arg to Gln
C 1379	248	CGG to CAG	Arg to Gln
C 1192	248	CGG to CAG	Arg to Gln
C 963	248	CGG to TGG	Arg to Trp
C 1041	248	CGG to TGG	Arg to Trp
C 1061	248	CGG to TGG	Arg to Trp
C 1376	248	CGG to TGG	Arg to Trp
C 1365	267	CGG to CCG	Arg to Pro
C 904	272	GTG to ATG	Val to Met
C 1106	273	CGT to TGT	Arg to Cys
C 848	273	CGT to TGT	Arg to Cys
C 960	273	CGT to TGT	Arg to Cys
C 1045	273	CGT to CAT	Arg to His
C 1138	273	CGT to CAT	Arg to His
C 974	273	CGT to CAT	Arg to His
C 1168	273	CGT to CAT	Arg to His
C 1366	273	CGT to CAT	Arg to His
C 883	277	TGT to GGT	Cys to Gly
C 975	278	CCT to CGT	Pro to Arg
C 1356	278	CCT to CAT	Pro to His
C 941	282	CGG to TGG	Arg to Trp
C 1107	282	CGG to TGG	Arg to Trp
C 1131	282	CGG to TGG	Arg to Trp
C 1151	127–128	TCC CCT to TCCCT	1-bp deletion
C 1167	129–132	GCC CTC AAC AAG to GCAG	8-bp deletion
C 1397	189–191	GCC CCT CCT to GCCCCT	3-bp deletion
C 1125	199–200	GGA AAT to GGAAT	1-bp deletion
C 966	200–201	AAT TTG to AATTG	1-bp deletion
C 1350	208–209	GAC AGA to GACA	2-bp deletion
C 1263	208–209	GAC AGA to GACA	2-bp deletion
C 1110	209–215	AGA . . . AGT to T	20-bp deletion
C 953	216–217	GTG GTG to GTGGTGGTGTG	5-bp insertion
C 1391	239–240	AAC AGT to AACT	2-bp deletion
C 1088	241–242	TCC TGC to TCTGC	1-bp deletion
C 1342	274–275	GTT TGT to GTTT	2-bp deletion
C 850		Deletion of part of exon 5; sequence alteration not clear	
C 1028		Detected by CDGE in exon 6	
C 968		Detected by CDGE in exon 5	
C 1010		Detected by CDGE in exon 6	
C 1073		Detected by CDGE in exon 5	

in rectal cancers than in tumors at other sites ($P = 0.029$). *TP53* mutations occurred significantly more frequently in aneuploid tumors than in diploid ones ($P < 0.001$). Mutations in *TP53* seemed to be inversely associated with MIN status ($P = 0.028$), and LOH of 17p13 was found more frequently in tumors with *TP53* mutations than in tumors without such mutations ($P < 0.001$).

Each of the 105 mutations was found by CDGE, and the specific sequence alterations were identified in 100 of them (Table 2). In one sample (C 850), the exact sequence of the deletion seen in exon 5 was difficult to interpret. In four samples, the mutated band on CDGE was weak but clear, but the sequence alteration could not be determined possibly due to the low amount of mutated cells in these samples (36). Three samples contained two different mutations each: one had two missense mutations, and each of the other two had a deletion and a missense mutation. In a total of 78 missense mutations, 7 nonsense mutations, 14 deletions, 1 inversion, and 1 insertion were found. No significant differences in the distribution of missense/nonsense mutations *versus* deletions were seen for any of the clinicopathological variables analyzed. Males tended to have a slightly higher frequency of deletions than did females (20 *versus* 9%), and aneuploid tumors tended to have a higher frequency of missense and nonsense mutations than did diploid tumors (89 *versus* 75%). These tendencies did not reach statistical significance. Seventy-eight % of the missense and nonsense mutations were transitions and 22% transversions. Transversions seemed to be more frequent in tumors located with left-sided location (44%) *versus* those right-sided location

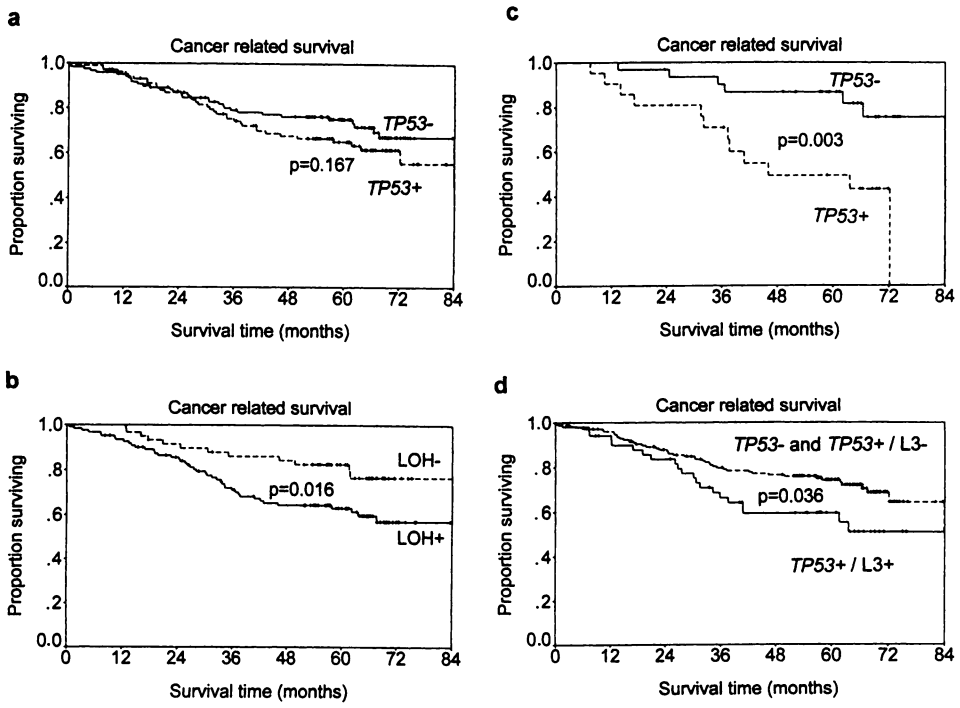

Fig. 2 Survival analyses (cancer-related Kaplan-Meier plots) of CRC patients in relation to different variables. *a*, total cohort in relation to *TP53* mutation status; $n = 120$ with *TP53* wild type (*TP53*−), and $n = 102$ with TP53 mutation (*TP53*+). *b*, total cohort in relation to LOH at 17p13; $n = 59$ with LOH− (wild type), and $n = 121$ with LOH+. *c*, patients with left-sided tumors in relation to *TP53* mutation status; $n = 31$ with *TP53*−, and $n = 21$ with *TP53*+. *d*, total cohort in relation to site of the *TP53* mutations; $n = 50$ cases with mutations affecting the L3 domain of the p53 protein (*TP53*+/*L3*+), $n = 48$ cases with mutations not affecting the L3 domain of the p53 protein (*TP53*+/*L3*−), and $n = 120$ cases with no detectable *TP53* mutation (*TP53*−).

(10%) and rectal tumors (18%; $P = 0.039$). Most (64%) of the transitions were G-to-A changes, and 83% of these were found at CpGs. No significant difference was seen in the type of mutations with respect to any clinicopathological variables analyzed, although G-to-T transversions were five times more frequent in males than in females.

The spectrum of missense mutations is shown in Fig. 1*b* and is similar to that reported in the literature database (39), with codons 175, 245, 248, and 273 being the four most common altered sites.

The survival of patients with mutations in the *TP53* gene was not significantly different from that of patients without mutations (Fig. 2*a*). The effect of LOH at 17p13 on cancer-related survival is illustrated in Fig. 2*b* and shows a significant difference ($P = 0.016$). The effect of LOH seemed to be restricted to the stratum of patients with no mutations of *TP53* ($P = 0.018$). In patients with *TP53* mutations, no difference was seen between presence and absence of LOH. A significantly shorter survival was observed in patients with left-sided tumors and the presence of a *TP53* mutation compared to those with left-sided tumors who did not have a mutation (Fig. 2*c*).

No difference in survival was seen between patients with missense mutations *versus* those with frameshift mutations. The mutations were classified into three groups according to possible different biological functions: (*a*) severe flexible mutants (codons 175, 176, 179, 238, 245, 267, 172, 173, and 181) and severe contact mutants (codons 248 and 282), (*b*) scrambled mutants (codons 196, 203, 205, and 216), and (*c*) mild DNA contact mutants (273 and 277) and mild flexible mutants (codon 272). Comparison of survival among these groups revealed no statistically significant differences. When the mutations were stratified according to the different domains, patients with mutations affecting the L3 loop of the p53 molecule had a significantly shorter cancer-related survival than did patients with other mutations or no mutations (Fig. 2*d*).

Patient survival is significantly associated with Dukes' stage ($P < 0.0001$, log rank test for trend; data not shown). Stratified log rank tests of the variables above with Dukes' stage as a stratification factor gave results similar to those of the log rank tests presented above.

Patients with diploid tumors seem to do better than patients with aneuploid tumors regarding cancer-related survival ($P = 0.05$). Stratification for ploidy in the analyses of mutation type (*i.e.*, mutations affecting different domains, as analyzed in Fig. 2*d*) gave a slightly reduced effect ($P = 0.07$). Stratification for other variables in the analyses of LOH produced results similar to those of log rank tests without stratification.

The analyses of overall survival for the variables described above gave results similar to those for cancer-related survival, although slightly less significant.

DISCUSSION

The presence of a *TP53* mutation has been associated with poor prognosis in CRC patients in several previous studies (6–9), but contradictory findings have also been reported (10, 11). In this study, a difference in survival rate was seen, although it was not statistically significant. A total of 222 patients have been included, and among these are 71 cancer-related deaths. Thus, differences in survival proportions between groups of patients need to be of a size 15–20% or larger to achieve acceptable power (>80%) to be detected at the 5% significance level. A possible explanation for the discrepancy between the previous studies may be differences in the compo-

sition of the patient cohorts with respect to tumor location and Dukes' stage. In this study, the patients with a tumor located to the left side had a significant shorter survival if a *TP53* mutation was present compared to those who did not have a mutation. This indicates that both the etiology and the tumorigenic pathway are different regarding the site of the tumor. Also, treatment differences may contribute to the observed differences. The mechanism by which mutation of the *TP53* gene is associated with poorer patient survival is as yet unclear. Mutated p53 proteins have lost their ability to function in cell cycle arrest, apoptosis, inhibition of tumor growth, and preservation of genetic stability. Thus, an aggressive tumor with selective growth advantage, accumulating additional genetic alterations as well as conferring resistance to radio- or chemotherapy, may be the result of a *TP53* mutation.

Hazards for different groups of patients seemed far from proportional for most variables studied, and the simultaneous effect of different mutations and clinicopathological variables on survival has not been studied. Stratified log rank tests showed that the effect of LOH at 17p13 was only slightly altered by stratification for ploidy, mutation of *TP53*, or mutation affecting zinc binding. However, the distribution of mutation of *TP53* and LOH at 17p13 differs between patients with and without mutations of the zinc-binding domain, and stratification for ploidy or LOH reduces the effects of these mutations.

Many *P*s have been calculated without hypotheses specified prior to the statistical analyses. Such *P*s carry a much reduced weight of interference, and we have chosen to pay attention to *P*s below 0.001. Considering the three variables of primary interest, the commonly chosen significance level of 5% was maintained. The present results should probably be viewed as hypotheses generating rather than conclusive.

In tumor suppressor genes such as *TP53*, a point mutation in one allele is often coupled to allelic loss of the other allele, as confirmed in the present study. However, a higher frequency of allele loss than mutations was seen, and patients with LOH at 17p13 in their tumors had a significantly worse prognosis than patients without LOH. The likelihood that the CDGE analyses have missed mutations within the screened area is low. The sensitivity of this technique to detect mutations even if only present in a low amount of the tumor cells is more than 99% (38). Therefore, our results indicate that mutations outside the screened area (exons 5–8) could be present in tumors with LOH. However, very few such mutations have been reported in colorectal carcinomas (39), but this might be biased, given that most studies have only examined exons 5–8. This study confirms that aneuploidy is associated with the presence of a *TP53* mutation and with poor prognosis. A previous study has also shown that mutations in the *TP53* gene precede aneuploidy in colorectal carcinomas (40).

The finding of an inverse relationship between presence of MIN and presence of a *TP53* mutation is in agreement with other reports (41, 42). These data support the hypothesis that mismatch repair deficiency provides a p53-independent pathway for development of CRCs.

The spectrum of mutations observed in the present cohort of patients does not differ significantly from that reported in the literature (39), giving no evidence of large differences in environmental carcinogen exposure. The higher frequency of transversion in left-sided and rectal tumors found in this study indicates that carcinogen exposure may be more prominent for the development of these tumors than for the tumors located on the right side of the colon.

The high frequency of *TP53* mutations distributed over a large region of the molecule has led to the speculation that different mutations have different biological and biochemical properties. It has been observed that not all mutations are functionally equivalent. Specific mutations may contribute to a more aggressive tumor and perhaps to a tumor resistant to radio- and chemotherapy. *In vitro* studies have shown heterogeneous biological effects induced by different mutations (43–45). Previous studies on breast cancer have shown that mutations in the zinc-binding domain (L2 and L3) are associated with poor prognosis (31) and that mutations particularly affecting the L3 domain of the protein confer resistance to doxorubicin treatment (32). In the present study of CRC patients, mutations affecting the L3 domain gave a significantly shorter survival. This indicates that mutations disrupting this domain contribute not only to chemoresistance but also to a more aggressive tumor with growth advantages. L3, depending almost exclusively on arginine 248, is responsible for the contacts between the p53 protein and the minor groove of target DNA. Total loss of DNA-binding capacity is expected for mutations of arginine 248. In contrast, the contacts between p53 and the major groove of target DNA involve several residues (residues 273, 277, 280, 281, 283, and 120; see Fig. 1*a*). Mutations of any of these residues may not be sufficient to completely disrupt the binding of p53 inside the major groove. The codon 175 resides in the L2 domain. Mutations affecting L2 and particularly codon 175 were, in this study, found not to be associated with shorter survival. This is in contrast to a recent study (7), in which mutations of codon 175 gave particularly more aggressive tumors with a significantly shorter survival. One explanation for this discrepancy may be that in our study, of the 11 patients with mutations in codon 175, 7 were of Dukes' stage A or B and 4 were of Dukes' stage C or D, whereas in the previous study, only 1 of 11 patients was of Dukes' stage A or B.

The results from this study and others show that mutations in the *TP53* gene play a pivotal role in determining the biological behavior of colorectal carcinomas, as well as that of other solid tumors. Some mutations may modify the effectiveness of postoperative therapies and thereby be more important than others. Additional studies are needed to clarify these issues. Knowledge of the *TP53* status of a tumor, with respect to the presence of a mutation, the specific nature of the lesion, and the presence/absence of a wild-type allele in addition to the mutated one, may be required to more precisely predict both the disease course and the response to different postoperative therapeutic interventions, especially those based on the induction of apoptosis. In new trials evaluating different drugs in postoperative CRC treatment, determination of the *TP53* status of the tumor should be included.

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