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Prognostic significance of *TP53* gene mutation in 995 cases of colorectal carcinoma: influence of tumour site, stage, adjuvant chemotherapy and type of mutation

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Abstract

Previous studies on the prognostic significance of *TP53* gene alterations in colorectal cancer (CRC) have led to conflicting results. The present study investigated the prognostic significance of *TP53* gene mutation in a very large series of 995 Dukes' B and C CRC patients, the majority of whom did not receive chemotherapy. Mutations were found in 385 (39%) cases and were not associated with tumour stage, histological grade, patient age or sex. Significantly more mutations were found in tumours from the left-sided colon compared with those from the right side (43% versus 34%, $P=0.006$). *TP53* gene mutation had no prognostic value in the overall series or in different site or stage subgroups. None of the different types of *TP53* gene mutation showed prognostic value. A trend for association with worse survival was observed in the patient subgroup that received adjuvant chemotherapy (Hazard Ratio (HR) 1.4, 95% confidence interval (CI) 0.89–2.21, $P=0.15$). These results indicate that mutation of the *TP53* gene is not a useful prognostic marker for CRC patients who do not receive adjuvant chemotherapy. Further study is required to determine whether different types of *TP53* mutation might be of value in predicting the response of CRC patients to chemotherapy.

Keywords: Colorectal carcinoma; *TP53*; Prognostic marker; Mutation

1. Introduction

Colorectal cancer (CRC) is the fourth leading cause of cancer death worldwide and the fourth most commonly occurring cancer with an estimated 875 000 new cases being diagnosed in 1996 [1]. Despite significant advances in both surgical methodology and adjuvant therapy regimes, long-term survival for CRC patients remains in the range of 50–60%. Considerable interest has therefore focused on the identification of novel tumour-based markers that can more accurately predict the course of the disease, as well as help to determine optimal adjuvant therapy approaches.

One of the most intensively studied tumour markers is the *TP53* tumour suppressor gene. This gene encodes for a 53 kDa phosphoprotein and is frequently targeted for inactivation in a wide range of tumours [2]. It is the target of point mutations and of small deletions and insertions that lead to total or partial inactivation of protein function. Inactivation is believed to abolish the ability of *TP53* to maintain genomic integrity through regulation of various activities including control of cell cycle arrest, DNA repair and apoptosis.

In some tumour types, notably breast cancer, mutation of the *TP53* gene is consistently associated with shortened patient survival [3]. The significance of *TP53* mutation as a prognostic marker for CRC is still a matter of controversy. Two groups have reported strong associations between *TP53* mutation and poorer prognosis in cohorts of more than 200 patients [4,5], although both found the association was confined to distal tumours. However, other studies have reported no association between *TP53* mutation and patient survival [6–8]. There has also been a lack of consensus regarding

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the prognostic significance of specific types of *TP53* mutations. Goh and colleagues reported that mutations in the evolutionarily conserved domains of *TP53* were associated with worse prognosis than those outside these domains [9]. In contrast, Kressner and colleagues recently reported that mutations in the non-conserved regions had the worst prognosis [10]. Additionally, Borresen-Dale and colleagues found that mutations affecting the L3 zinc-binding domain had a significantly shorter cancer-related survival [4]. These discrepancies may be related to the use of different mutation screening methods, as well as to the composition of the tumour series investigated, particularly with regard to tumour site, stage and the use of adjuvant therapies.

In the present study, we have addressed the dual issues of the prognostic significance of *TP53* mutation in different CRC subgroups and of different types of *TP53* mutation. We screened 995 colorectal tumours using similar polymerase chain reaction–single strand conformation polymorphism (PCR–SSCP) methodology with which we previously observed strong associations between *TP53* mutation and poor survival in breast [11,12] and gastric cancers [13].

2. Patients and methods

2.1. Characteristics of patients and tumours

Dukes' B or C stage CRC from 995 patients undergoing surgery for their disease at the Sir Charles Gairdner Hospital, Nedlands, between 1985 and 1997 were identified from histopathological records. These cases were from a previously analysed tumour series in our laboratory [8,14,15]. There was an over-representation of Dukes' C stage (71%) due to their selection in two of the studies [14,15]. Right-sided tumours were classified as originating in the proximal or transverse colon and left-sided tumours as originating in the descending colon or rectum. Information on the major clinicopathological features of the tumour series is shown in Table 1. Information on tumour site and histological grade was not available for 31 (3%) and 41 (4%) cases because of incomplete pathological reporting. The median age of the patients was 69.5 years (range: 19–100) and the median follow-up time was 102 months (range: 33–178 months). Information on patient survival was obtained from the Western Australian Health Department death registry. At the end of the study period, 493 (50%) of patients had died due to a recurrence of their disease. Patients who died of unrelated causes were censored from the survival analysis at the time of death. 33 patients died perioperatively and were excluded from the survival analyses. Information on adjuvant therapy was available for 924 (93%) cases. Of these, 157 (17%) received chemotherapy with curative intent. In the large majority of cases (85%) this consisted of six-monthly cycles of intravenous 5-fluorouracil and oral levamisole. The 71 cases (7%) with unknown chemotherapy status were all from the pre-1991 period when almost none of the patients received adjuvant treatment.

2.2. PCR–SSCP screening and sequencing of TP53 gene mutations

Screening for mutations in exons 4–10 of the *TP53* tumour suppressor gene was carried out using both isotopic and non-isotopic PCR–SSCP as previously described [12,16]. Results for exons 5, 7 and 8 containing the conserved region sequences and mutation hotspots were obtained in all 995 cases. Exons 4, 9 and 10 were screened in 487 (49%), 75 (8%) and 107 (11%) tumours respectively. Due to the relatively low incidence of mutation found in these exons (5, 0 and 0.3%, respectively) and limits on resources, screening of the entire tumour series was not carried out. Exon 6 was screened in 735 cases (74%). This exon contains no mutation hotspots or conserved region sequences and therefore mutations were not further identified by sequencing.

Tumour samples showing aberrantly migrating bands in two or more independent PCR–SSCP runs were considered to contain a mutation. The majority of mutations (281/347, 81%) detected in exons 5, 7 and 8

Table 1
Association of *TP53* gene mutation with clinicopathological features

Feature	*n* (%)	*TP53* mutation *n* (%)	*P* value
All tumours	995 (100)	385 (39)	
Dukes' stage			
B	293 (29)	115 (39)	
C	702 (71)	270 (38)	$P=0.816$
Site			
Right	361 (36)	122 (34)	
Left	603 (61)	258 (43)	$P=0.006$
Information unavailable	31 (3)		
Grade			
Well	120 (12)	49 (41)	
Moderate	626 (63)	251 (40)	
Poor	208 (21)	73 (35)	$P=0.233$
Information unavailable	41 (4)		
Age (years)			
<69.5	486 (49)	179 (37)	
$\geqslant 69.5$	509 (51)	206 (40)	$P=0.239$
Sex			
Male	477 (48)	187 (39)	
Female	518 (52)	198 (38)	$P=0.751$
Chemotherapy			
No	767 (77)	309 (40)	
Yes	157 (16)	55 (35)	$P=0.220$
Information unavailable	71 (7)		

were further characterised by either DNA sequencing or hotspot identification. Mutant template for DNA sequencing was obtained using either one of two methods. Initially, aberrantly migrating bands suspected of containing a mutation were excised, the DNA eluted from the gel slice and re-amplified in a sequencing reaction as previously described [16]. The bandstab technique [17] was also utilised. This involved stabbing the mutant band with a sterile micropipette tip and placing it in 50 μl of standard PCR reaction mix for 1–2 min at room temperature prior to amplification for 30 cycles. The PCR product was then purified using the QIAquick PCR Purification Kit (QIAGEN, Melbourne, Australia) and used as a template for direct sequencing with the ABI Prism automated DNA sequencing kit (Perkin-Elmer, Melbourne, Australia). A proportion of the *TP53* mutations suspected of being hotspots because of their distinctive SSCP banding pattern were identified by running alongside positive controls known to contain these mutations [8]. In some of the tumours with aberrantly migrating SSCP bands that were not sequenced, these may have been due to polymorphisms or silent mutations. However, we believe these to have occurred at a very low frequency since none was found in the large number of cases that were confirmed by sequencing.

2.3. *Definition of* TP53 *mutation types*

Kaplan–Meier survival analysis was conducted for various *TP53* mutations grouped according to the site of mutation or the possible functional effect of that mutation. These groups included the particular exon in which the *TP53* mutation occurred, mutations within the hotspot codons 175, 245, 248, 273 and 282 [18], denaturing mutations that directly affect the stability of p53 (codons 143, 175, 245, 249, 282), mutations in the zinc binding codons 176, 179, 238 and 242 [19], mutations that affect the ability of p53 to bind to DNA (codons 120, 241, 248, 273, 276, 277, 280, 281 and 283) [19], mutations that occur within the L2 and L3 loops (codons 163–195 and 236–251) and thereby affect the tertiary conformation of p53 [19], mutations that occur in the evolutionarily conserved regions of the *TP53* core domain (codons 117–142, 171–180, 234–258 and 270–287) [20] and mutation type, specifically whether it was a single base substitution or a deletion/insertion, and transition or transversion single base substitutions.

2.4. *Statistical analysis*

The χ^2 test was used to determine associations between *TP53* gene mutation and the various clinicopathological features of tumours. The Mantel–Haenszel test for linear association was used to determine correlation with histological grade, treated as a continuous variable. Univariate survival analysis was carried out using the method of Kaplan–Meier and differences between survival curves were compared using the log-rank test. Only cases with single mutations were considered for survival analyses. All tests were two-tailed and statistical significance was assumed when $P < 0.05$. Analyses were carried out using the Statistical Package for the Social Services (SPSS) software package (Chicago, IL, USA).

3. Results

A total of 398 mutations in the *TP53* gene were found in 385 of the 995 (39%) tumours. Thirteen tumours contained two different mutations. The numbers of mutations detected in exons 4, 5, 6, 7, 8 and 10 were 18 (5%), 131 (33%), 32 (8%), 116 (29%), 100 (25%) and one (0.3%), respectively. With the exception of exons 4 and 10, the distribution of mutations amongst the different exons was similar to that of the International Agency for Research on Cancer (IARC) database [21] of *TP53* gene mutations in CRC (Fig. 1a). The apparently higher proportion of exon 4 and 10 mutations

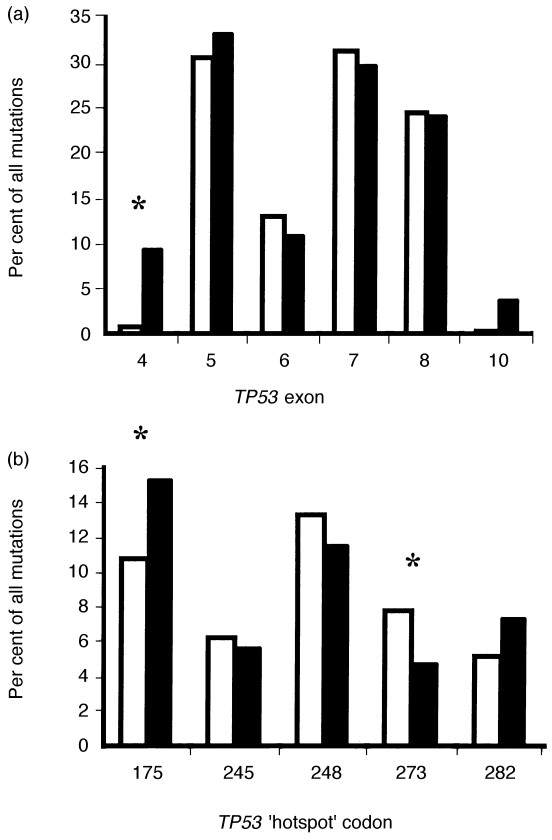

Fig. 1. (a) Comparison of exon distribution of *TP53* gene mutations between International Agency for Research on Cancer (IARC) database (blank columns) and the present study (filled columns). (b) Comparison of *TP53* 'hotspot' mutations between the IARC database (blank columns) and the present study (filled columns). *Significant difference between results (χ^2-test).

found in the present study is probably because most earlier studies did not screen these two exons. Mutations in the hotspot codons 175, 245, 248, 273 and 282 accounted for 43% of all mutations in both the present study (172/398) and in the IARC database. We observed a significantly higher proportion of codon 175 mutations, but a significantly lower proportion of codon 273 mutations (Fig. 1b) in comparison with the IARC database. A list of all the mutations identified and the resulting amino acid sequence changes are shown in Table 2.

Associations between *TP53* gene mutation and the standard clinicopathological features for CRC are shown in Table 1. No associations were observed for tumour stage, histological grade, patient age or sex, however, mutations were significantly more frequent in

Table 2
Characterisation of 398 *TP53* gene mutations[a] detected in 385 colorectal carcinomas

Codon	*n*	Nucleotide change	Amino acid change	Codon	*n*	Nucleotide change	Amino acid change
135	1	tgc-tgg	cys-trp	231	1	1 base pair deletion	
135	1	tgc-tac	cys-tyr	234	1	3 base pair deletion	
138	1	ggc-gtc	ala-val	237	1	atg-atc	met-ile
141	1	tgc-cgc	cys-arg	240	1	agt-ggt	ser-gly
141	1	tgc-tac	cys-tyr	242	1	3 base pair insertion	
145	1	ctg-ccg	leu-pro	244	3	ggc-gac	gly-asp
145	1	ctg-gtg	leu-val	244	1	ggc-tgc	gly-cys
146	1	tgg-tag	trp-stop	244	1	ggc-gtc	gly-val
147	1	8 base pair insertion		244	1	1 base pair deletion	
150	1	1 base pair deletion		245	1	ggc-cgc	gly-arg
151	1	ccc-cgc	pro-arg	245	1	ggc-gac	gly-asp
151	2	ccc-tcc	pro-ser	245	1	ggc-tgc	gly-cys
151	2	ccc-acc	pro-thr	245	17	ggc-agc	gly-ser
151	2	1 base pair insertion		245	2	ggc-gtc	gly-val
151	1	1 base pair deletion		248	22	cgg-cag	arg-gln
152	1	ccg-cgg	pro-arg	248	22	cgg-tgg	arg-trp
152	2	ccg-ctg	pro-leu	249	1	agg-agt	arg-ser
152	2	1 base pair insertion		250	1	ccc-ctc	pro-leu
152	2	1 base pair deletion		251	1	atc-aac	ile-asn
153	1	1 base pair insertion		251	1	9 base pair deletion	
153	1	8 base pair insertion		254	1	3 base pair deletion	
155	1	acc-aac	thr-asn	259	1	1 base pair insertion	
156	1	1 base pair deletion		265	1	ctg-ccg	leu-pro
158	2	cgc-cac	arg-his	266	1	10 base pair insertion	
158	1	13 base pair deletion		272	1	gtg-ctg	val-leu
160	1	cgg-cag	arg-gln	272	3	gtg-atg	val-met
161	1	gcc-acc	ala-thr	273	18	cgt-cat	arg-his
165	1	cag-tag	gln-stop	274	1	gtt-ctt	val-leu
167	1	cag-tag	gln-stop	275	1	tgt-ttt	cys-phe
171	1	6 base pair deletion		275	2	tgt-tat	cys-tyr
172	1	gtt-ttt	val-phe	276	2	gcc-tcc	ala-ser
173	3	gtg-ttg	val-leu	277	1	tgt-tga	cys-stop
173	1	gtg-ctg	val-leu	278	2	cct-gct	pro-ala
173	1	gtg-atg	val-met	278	1	cct-cgt	pro-arg
173	1	1 base pair deletion		278	1	cct-ctt	pro-leu
175	2	cgc-tgc	arg-cys	278	1	cct-tct	pro-ser
175	1	cgc-ggc	arg-gly	278	2	cct-act	pro-thr
175	56	cgc-cac	arg-his	280	1	aga-aca	arg-thr
176	3	tgc-ttc	cys-phe	281	1	gac-ggc	asp-gly
176	1	tgc-tcc	cys-ser	282	1	cgg-ccg	arg-pro
177	1	1 base pair deletion		282	27	cgg-tgg	arg-trp
177	3	18 base pair deletion		282	1	18 base pair deletion	
179	1	cat-aat	his-asn	283	1	cgc-cac	arg-his
179	2	cat-tat	his-tyr	283	1	20 base pair deletion	
180	1	gag-aag	glu-lys	285	2	gag-aag	glu-lys
181	1	cgc-cac	arg-his	286	1	gaa-gca	glu-ala
183	1	tca-tga	ser-stop	286	1	gaa-gga	glu-gly
214	1	cat-cag	his-gln	294	4	gag-tag	glu-stop
225	1	6 base pair insertion		294	1	1 base pair deletion	

[a] 18 exon 4, 14 exon 5, 31 exon 6, 32 exon 7, 20 exon 8 and one exon 10 mutations were not sequenced. $n = 282$ were sequenced.

tumours arising in the left-sided colon and rectum. The frequency of mutations in left- and right-sided CRC observed here was compared with previous studies that reported site-specific results for *TP53* mutation (Table 3). Of 13 studies showing this information, five found significantly more mutations in the left compared with right colon. More frequent *TP53* mutations in the left colon were also observed in another four studies, but the difference was non-significant, presumably due to the smaller number of cases examined. Four studies found a similar incidence of *TP53* mutation in the left and right colon. Data from all studies were combined for comparison with the present results (Table 3). A significantly higher incidence of *TP53* mutation was apparent in left- compared with right-sided colon tumours (52% versus 37%, $P < 0.001$, χ^2-test). There was no significant difference in the incidence of *TP53* mutation in right-sided colon tumours between our study and previous studies, however, we observed fewer mutations in left-sided colon tumours (43% versus 52%, $P < 0.001$, χ^2-test).

Univariate survival analysis revealed that tumour stage and histological grade were significant prognostic factors for patient survival in this series, but not the features of tumour site, patient age or sex (Table 4). *TP53* mutation showed no significant prognostic value in the overall series, in site or stage subgroups, or in patients who did not receive chemotherapy (Table 4). Interestingly, in the relatively small group of patients who received chemotherapy, a trend for worse survival was seen in cases with *TP53* mutation. Survival analysis was also carried out for patients with the various types of *TP53* mutation as described earlier and compared with the survival of those with wild-type *TP53* (Table 5). No significant differences were observed for any of the mutation groups, although trends for worse survival were seen in patients with mutations in exon 8 or in the codon 282 hotspot contained within this exon.

4. Discussion

TP53 gene mutations are consistently associated with shortened patient survival in breast cancer [3]. In contrast, studies on the prognostic significance of this genetic alteration in CRC have given conflicting results [2]. Possible reasons for discordant findings include the accuracy of techniques used to screen for mutations, the number of cases investigated, the length of patient follow-up, the clinical characteristics of the tumour series (particularly stage and site) and the use of adjuvant therapies. In an attempt to overcome these problems, we investigated the prognostic significance of *TP53* gene mutation in a very large series of Dukes' B and C CRC with long patient follow-up and with known adjuvant therapy status. Furthermore, we used similar SSCP mutation screening techniques with which we previously observed strong associations between *TP53* mutation and poor prognosis in breast [11,12] and gastric [13] carcinomas, thus allowing a direct comparison with these tumour types. The majority of mutations detected by SSCP were further characterised by DNA sequencing or hotspot identification, allowing us to also investigate the prognostic significance of various types of *TP53* gene mutation.

The relative distribution of *TP53* gene mutations among the various exons and mutation hotspots observed in this study compares favourably with that

Table 3
Comparison of studies ($n > 50$) that reported frequency of *TP53* gene mutation with respect to tumour site in colorectal cancer (CRC)

Author [Ref.]	*n*	Right tumours[a] *TP53* mutations/total (%)	Left tumours[b] *TP53* mutations/total (%)	*P* value[c]
Borresen-Dale [4]	222	24/67 (36)	78/155 (50)	<0.05
Bosari [6]	126	20/34 (59)	54/92 (59)	NS
El-Mahdani [22]	109	8/26 (31)	36/83 (43)	NS
Goh [5]	328	31/102 (30)	135/226 (60)	<0.001
Hamelin [23]	85	3/15 (20)	41/70 (59)	<0.010
Iniesta [24]	61	3/15 (20)	15/46 (33)	NS
Jernvall [25]	72	12/23 (52)	24/49 (49)	NS
Kressner [10]	189	28/78 (36)	69/111 (62)	<0.001
Leahy [7]	66	6/17 (35)	21/49 (43)	NS
Lleonart [26]	125	22/50 (44)	31/75 (41)	NS
Pauly [27]	72	2/16 (13)	14/56 (25)	NS
Tortola [28]	132	9/31 (29)	57/101 (56)	<0.01
Watatani [29]	108	18/30 (60)	45/78 (58)	NS
Total	1695	186/504 (37)	620/1191 (52)	<0.001
Present study	964	122/361 (34)	258/603 (43)	0.006

NS, non-significant.
[a] Proximal and transverse.
[b] Distal and rectal.
[c] χ^2-test.

Table 4
Cox proportional hazard univariate survival analyses of clinicopathological features and of *TP53* gene mutation

Feature	*n*	Hazard ratio (HR) (95% confidence interval (CI))[a]	*P* value
Dukes' stage	948	2.72 (2.16–3.44)	< 0.0001
Tumour site	919	1.16 (0.97–1.40)	0.11
Histological grade	907	1.52 (1.29–1.80)	< 0.0001
Age	948	1.15 (0.96–1.37)	0.13
Sex	948	1.00 (0.83–1.19)	0.97
TP53 mutation			
Total	948	0.96 (0.80–1.15)	0.62
Left colon	575	0.92 (0.73–1.15)	0.46
Right colon	341	1.07 (0.78–1.46)	0.67
Dukes' B	281	1.03 (0.67–1.57)	0.90
Dukes' C	665	0.94 (0.77–1.15)	0.57
No chemotherapy[b]	740	0.89 (0.73–1.10)	0.28
Chemotherapy[c]	154	1.40 (0.89–2.21)	0.15

[a] For each clinicopathological feature, the categories compared were the same as those shown in Table 1.
[b] Survival information was not available for 27 cases.
[c] Survival information was not available for 3 cases.

reported in the IARC database for CRC (Fig. 1a and b). Although we observed a similar incidence of *TP53* mutation in right-sided colon cancers to other workers (Table 3), the frequency in left-sided tumours was approximately 10% lower (43% versus 52%). The reason for this is unknown, but may be due to the inability of our SSCP method to detect some *TP53* mutations that are specific to tumours of the left colon. Another explanation is that the lower incidence is real and reflects a different aetiology of CRC in the Australian population. Although of a smaller magnitude, the different frequency of *TP53* mutation between left- and right-sided tumours observed here and in previous studies (Table 3) is in line with differences reported for several other genetic alterations. These include a 2- to 4-fold higher frequency of 17p, 18q and 5q allelic losses in left-sided tumours [30] and a 10-fold higher frequency of microsatellite instability in right-sided tumours [31].

While the present study (Table 4) found no prognostic significance for *TP53* mutations at either tumour site, two studies reported an association with worse survival in left- but not in right-sided tumours [4,5]. Some workers have also reported stage specificity for the prognostic significance of *TP53* mutation [5,7], but this was not observed here or in some earlier studies [32,33]. The introduction in many institutes over the past decade of adjuvant chemotherapy with curative or palliative intent for Dukes' C and D CRC, respectively, may be an additional confounding factor. Two studies have reported a worse outcome for patients receiving chemotherapy if they had *TP53* mutation [9,34]. A similar trend was seen in the present study for the relatively small patient group that received chemotherapy (Table 4). In contrast, no survival difference was observed in patients who did not receive this treatment. These findings suggest that *TP53* mutation has no prognostic value in colorectal cancer patients who do not receive adjuvant chemotherapy. However, *TP53* mutation may be able to identify patients who do not respond as well to chemotherapy as those with the wild-type gene. Proper evaluation of the predictive significance of this molecular alteration will require analysis of tumours from matched treated and untreated patient groups such as those found in clinical trials. A finding of no survival benefit from chemotherapy for patients with *TP53* mutation would call into question current management practices for this tumour type.

The second major aim of the present work was to evaluate the prognostic significance of various types of *TP53* mutation. Four previous studies have examined this in CRC, each reporting different results. Goh and coworkers [9] showed that mutations in the codon 175 hotspot or in the conserved regions of the gene were associated with worse survival. In contrast Kressner and colleagues [10] found that mutations in non-conserved regions confer worse survival. Iniesta and coworkers [24] reported that exon 7 mutations were associated with poor prognosis, although they analysed only a small number of tumours. Borresen-Dale and colleagues [4] performed a number of subgroup analyses with respect to mutation type and found that patients with mutations in the L3 domain had the poorest survival compared with all other patients. In the present study, we found no significant prognostic value for any of the different types of mutation examined (Table 5). A weak trend for worse survival was only seen in tumours with exon 8 mutations or with codon 282 hotspot mutations located within this exon. The observed prognostic value

Table 5
Kaplan–Meier survival analysis of colorectal cancer patients with different types of *TP53* gene mutation

TP53 mutation type[a]	*n*	Endpoint survival (%)	*P* value
Wild-type *TP53*	588	49	
Mutant *TP53*[b]	360	49	0.62
Exon 4	16	56	0.78
Exon 5	117	50	0.88
Exon 6	29	45	0.70
Exon 7	109	56	0.12
Exon 8	89	39	0.27
Codon 175	51	55	0.43
Codon 245	21	52	0.61
Codon 248	40	50	0.88
Codon 273	17	47	0.96
Codon 282	26	31	0.13
All hotspots	155	48	0.89
All non-hotspots	205	49	0.56
Denaturing[c]	107	50	0.68
Non-denaturing	177	47	0.78
DNA contact	61	49	0.80
Non-DNA contact	223	48	0.70
L2[c]	72	54	0.41
L3	74	53	0.41
L2/L3	146	53	0.27
Non-L2/L3	154	44	0.72
Conserved[c]	215	52	0.29
Non-conserved	69	38	0.29
Missense/nonsense[c]	228	48	0.79
Deletion/insertion	28	50	0.83
Transition[c]	185	49	0.79
Transversion	43	47	0.94

[a] All mutation subgroups are compared with patients with wild-type *TP53*.
[b] 38 cases with double mutations or with perioperative death were excluded from this analysis.
[c] Only mutations that were definitively identified as belonging to these subgroups were analysed.

of different types of *TP53* mutation may have been confounded, however, by the use of adjuvant chemotherapy in a proportion of cases. Because of the relatively small number of patients with a *TP53* mutation and who received chemotherapy ($n = 55$) we were unable to determine the influence of mutation type on survival in this subgroup.

We conclude that although different types of *TP53* mutation have no overall prognostic value in CRC patients, an evaluation of whether they are associated with differing responses to chemotherapy awaits analysis of larger patient groups. In view of recent calls to expand the use of adjuvant chemotherapy for more Dukes' B and D stage CRC patients, the identification of molecular markers that help to identify those likely to benefit from this treatment is highly desirable.

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