original article

Annals of Oncology 17: 842–847, 2006
doi:10.1093/annonc/mdl035
Published online 8 March 2006

Functional categories of *TP53* mutation in colorectal cancer: results of an International Collaborative Study

B. Iacopetta*, A. Russo*, V. Bazan, G. Dardanoni, N. Gebbia, T. Soussi, D. Kerr, H. Elsaleh, R. Soong, D. Kandioler, E. Janschek, S. Kappel, M. Lung, C.-S. S. Leung, J. M. Ko, S. Yuen, J. Ho, S. Y. Leung, E. Crapez, J. Duffour, M. Ychou, D. T. Leahy, D. P. O'Donoghue, V. Agnese, S. Cascio, G. Di Fede, L. Chieco-Bianchi, R. Bertorelle, C. Belluco, W. Giaretti, P. Castagnola, E. Ricevuto, C. Ficorella, S. Bosari, C. D. Arizzi, M. Miyaki, M. Onda, E. Kampman, B. Diergaarde, J. Royds, R. A. Lothe, C. B. Diep, G. I. Meling, J. Ostrowski, L. Trzeciak, K. Guzińska-Ustymowicz, B. Zalewski, G. M. Capellá, V. Moreno, M. A. Peinado, C. Lönnroth, K. Lundholm, X. F. Sun, A. Jansson, H. Bouzourene, L.-L. Hsieh, R. Tang, D. R. Smith, T. G. Allen-Mersh, Z. A. J. Khan, A. J. Shorthouse, M. L. Silverman, S. Kato & C. Ishioka

Università di Palermo, Department of Oncology, Palermo, Italy

Received 24 November 2005; revised 26 January 2006; accepted 31 January 2006

Background: Loss of *TP53* function through gene mutation is a critical event in the development and progression of many tumour types including colorectal cancer (CRC). *In vitro* studies have found considerable heterogeneity amongst different *TP53* mutants in terms of their transactivating abilities. The aim of this work was to evaluate whether *TP53* mutations classified as functionally inactive (≤20% of wildtype transactivation ability) had different prognostic and predictive values in CRC compared with mutations that retained significant activity.

Materials and methods: *TP53* mutations within a large, international database of CRC (n = 3583) were classified according to functional status for transactivation.

Results: Inactive *TP53* mutations were found in 29% of all CRCs and were more frequent in rectal (32%) than proximal colon (22%) tumours ($P < 0.001$). Higher frequencies of inactive *TP53* mutations were also seen in advanced stage tumours ($P = 0.0003$) and in tumours with the poor prognostic features of vascular ($P = 0.006$) and lymphatic invasion ($P = 0.002$). Inactive *TP53* mutations were associated with significantly worse outcome only in patients with Dukes' stage D tumours (RR = 1.71, 95%CI 1.25–2.33, $P < 0.001$). Patients with Dukes' C stage tumours appeared to gain a survival benefit from 5-fluorouracil-based chemotherapy regardless of *TP53* functional status for transactivation ability.

Conclusions: Mutations that inactivate the transactivational ability of *TP53* are more frequent in advanced CRC and are associated with worse prognosis in this stage of disease.

Key words: chemotherapy, colorectal cancer, mutation, prognosis, *TP53*, transactivational ability

introduction

The *TP53* tumour suppressor gene encodes a 393 amino acid transcription factor that is activated by a variety of cellular stresses including DNA damage. The activated TP53 protein binds to regulatory regions of downstream target genes to initiate a programme of cell cycle arrest, DNA repair, apoptosis and angiogenesis [1]. Loss of *TP53* function is therefore likely to be a critical event in tumourigenesis and approximately half of all human cancers show this alteration [2]. Extensive databases on *TP53* mutation reveal that more than 80% are missense mutations leading to the synthesis of stable, full-length protein [3]. The common missense mutations disrupt the ability of *TP53* protein to bind DNA and transactivate target genes.

A recent study investigated the functional activity of 2314 different p53 mutations in terms of their ability to transactivate several target genes in a yeast assay [4]. The most common *TP53* mutations in human tumours show a clear loss of transactivation activity, however more than 50% of the rarer mutations retain significant activity [5]. Follow-up studies did not find any correlation between the transcriptional activity of

*Correspondence to: B. Iacopetta, School of Surgery and Pathology, University of Western Australia – Queen Elizabeth II, Medical Centre, 6009, WA, Nedlans. Tel: +61-8-93462085; Fax: +61-8-93462416; E-mail: bjiac@meddent.uwa.edu.au; or Dr A. Russo, Section of Medical Oncology, Department of Oncology, Università di Palermo, Via del Vespro 127, 90127 Palermo, Italy. Tel: +39 091 6552500; Fax: +39 091 6554529; E-mail: lab-oncobiologia@usa.net

179 mutant *TP53* species and their ability to induce apoptosis *in vitro* [6]. These results demonstrate that *TP53* mutants are functionally heterogeneous, thus complicating the interpretation of prognostic and predictive data for this genetic alteration in cancer.

The 'CRC-p53' International Collaborative Study has assembled data on *TP53* mutations in 3583 colorectal cancer (CRC) patients from 25 different research groups [7]. This database allowed evaluation of the prognostic significance of *TP53* mutation in relation to factors such as tumour site in the large bowel and the use of adjuvant chemotherapy. In addition, the prognostic significance of different types of *TP53* mutation including hot-spots and conserved domains was also investigated. In the present study we examined the clinical significance of *TP53* mutations in the CRC-p53 database following their classification according to functional activity for transactivation as determined by *in vitro* assay [4].

materials and methods

CRC-p53 database

The CRC-p53 database contains information on 3583 CRC patients from 17 different countries, including *TP53* mutation status and survival [7]. Clinical and pathological data include patient age and gender, tumour site, stage and grade, lymphatic and vascular invasion, and treatment with adjuvant chemotherapy. Cases were divided into three groups according to site of the primary tumour (proximal colon, distal colon, sigmoid colon/rectal) due to increasing evidence for different CRC aetiology according to site of origin in the large bowel [8, 9]. Follow-up times (median and range) were 58 months (1–194), 61 months (1–173) and 61 months (1–235) for patients with proximal colon, distal colon and rectal tumours, respectively. Proximal colon tumours were defined as originating in the caecum, ascending colon or transverse colon. Distal colon tumours were from the splenic flexure and descending colon, while rectal tumours included the sigmoid colon and rectum.

functional categorisation of TP53 mutations

TP53 mutation status was determined using SSCP, DGGE, TGGE and direct DNA sequencing. DNA sequence information was available for 894 tumours found to have mutation. These were classified into four functional categories for transactivation activity as described earlier [5]. Compared with wildtype *TP53*, these demonstrated <10% activity (category 0), 11%–20% activity (category 1), 21%–50% activity (category 2) and >50% activity (category 3). The 'active *TP53*' group comprised wildtype *TP53* and mutation categories 2 and 3 (21%–100% activity), while the 'inactive *TP53*' group comprised mutation categories 0 and 1 (0%–20% activity).

statistical analyses

Statistical analyses were performed separately for each of the three subgroups of patients classified according to the site of tumour origin (proximal colon, distal colon, sigmoid colon/rectum). Associations between functional category of *TP53* mutation (active or inactive) and clinico-pathological variables were evaluated by the chi-squared test with Yates correction, where appropriate. Survival time was calculated from the date of surgery to the date of death (cancer-related causes) or last follow-up, with times censored for patients dying from causes unrelated to CRC or peri-operatively. Significant differences between survival curves were evaluated by the log-rank and Wilcoxon tests or a test for trend where appropriate. Because of the multiple statistical analyses performed, only P values <0.01 were considered significant. All P values were two-sided.

results

A total of 2867 CRC cases were classified as having transcriptionally active (2048, 71%) or inactive (819, 29%) *TP53* according to the definition of Soussi et al. [5]. Meta-analysis of *TP53* mutations in CRC shows that all studies included in the CRC-p53 database have a normal distribution for loss of *TP53* function [5]. Associations between *TP53* mutation functional categories and clinicopathological features of CRC arising at different sites in the large bowel are shown in Table 1. A significantly higher frequency of inactive *TP53* mutations was seen in rectal (32%) compared with proximal colon (22%) cancers ($P < 0.001$), with distal colon cancers showing an intermediate frequency (26%). For each site, the more advanced Dukes' D tumours contained higher frequencies of inactive *TP53* compared with Dukes' A–C tumours. The association approached significance only in the proximal tumours ($P < 0.05$) but was highly significant in the overall CRC cohort ($P = 0.0003$). Vascular and lymphatic invasion by tumour cells are both indicators of more aggressive disease. Similar to advanced stage, tumours with these features showed higher frequencies of inactive *TP53* mutations. This was observed at each site but reached significance only in the overall CRC cohort ($P = 0.006$ and $P = 0.002$, respectively). No associations were seen between the functional status of *TP53* mutations and patient age or histological grade. A gender difference in the frequency of inactive *TP53* mutations was observed for distal colon tumours but not for the other sites. Mucinous tumours at each site showed a lower frequency of inactive *TP53* mutation but this reached significance only in the overall group ($P = 0.003$).

Inactive *TP53* mutations were examined for prognostic significance in the overall cohort and in all site and stage subgroups using active *TP53* status as the reference group (Table 2). No prognostic significance was seen for inactive *TP53* mutations in Dukes' A–C tumours from any of the sites or in the overall cohort. However, the inactive *TP53* mutations were associated with significantly worse patient survival for Dukes' D tumours from all sites as well as those from the proximal colon and rectum. Using wildtype *TP53* status as the reference group, the inactive *TP53* mutation group again showed prognostic significance only in Dukes' D tumours in the overall cohort (RR = 1.67, 95%CI 1.21–2.29, $P = 0.002$) as well as in the proximal colon (RR = 2.03, 95%CI 1.10–3.76, $P = 0.024$) and rectal (RR = 1.65, 95%CI 1.09–2.51, $P = 0.018$) tumour groups. The small number of active *TP53* mutations ($n = 60$) precluded separate analysis for the prognostic significance of this group against wildtype TP53.

The predictive value of *TP53* functional status for survival benefit from adjuvant 5-fluorouracil-based chemotherapy was investigated in Dukes' C patients (Table 3). For all three tumour sites and for the overall cohort, both patient groups with active and inactive *TP53* mutation status showed better outcome when treated with chemotherapy. The wildtype *TP53* group also showed better survival with the use of chemotherapy ($P < 0.001$ for the overall group), however the number of active *TP53* mutant cases was insufficient to allow separate analysis. The results indicate a similar level of survival benefit from adjuvant treatment regardless of the *TP53*

Table 1. Associations between functional categories of *TP53* mutation and clinicopathological features of CRC

Feature (*n*)	Active (%)	Inactive (%)	*P*
Proximal colon (851)	666 (78)	185 (22)	
Age <50 years (65)	52 (80)	13 (20)	
Age 50–75 years (560)	441 (79)	119 (21)	
Age >75 years (224)	173 (77)	51 (23)	NS
Male (412)	315 (76)	97 (24)	
Female (439)	351 (80)	88 (20)	NS
Dukes' A (44)	40 (91)	4 (9)	
Dukes' B (322)	253 (79)	69 (21)	
Dukes' C (421)	330 (78)	91 (22)	
Dukes' D (62)	42 (68)	20 (32)	NS (0.038)
Histological grade G1 (107)	92 (86)	15 (14)	
Histological grade G2 (515)	397 (77)	118 (23)	
Histological grade G3 (201)	162 (81)	39 (19)	NS
Non-mucinous (199)	143 (72)	56 (28)	
Mucinous (41)	34 (83)	7 (17)	NS
No vascular invasion (136)	104 (77)	32 (23)	
Vascular invasion (38)	26 (68)	12 (32)	NS
No lymphatic invasion (110)	87 (79)	23 (21)	
Lymphatic invasion (99)	65 (66)	34 (34)	NS (0.029)
Distal colon (282)	208 (74)	74 (26)	
Age <50 years (28)	19 (68)	9 (32)	
Age 50–75 years (188)	145 (77)	43 (23)	
Age >75 years (64)	44 (69)	20 (31)	NS
Male (132)	107 (81)	25 (19)	
Female (149)	101 (68)	48 (32)	NS (0.011)
Dukes' A (23)	17 (74)	6 (26)	
Dukes' B (119)	93 (78)	26 (22)	
Dukes' C (115)	83 (72)	32 (28)	
Dukes' D (25)	15 (60)	10 (40)	NS
Histological grade G1 (47)	34 (72)	13 (28)	
Histological grade G2 (189)	143 (76)	46 (24)	
Histological grade G3 (36)	24 (67)	12 (33)	NS
Non-mucinous (91)	63 (69)	28 (31)	
Mucinous (13)	11 (85)	2 (15)	NS
No vascular invasion (80)	51 (64)	29 (36)	
Vascular invasion (8)	3 (38)	5 (62)	NS
No lymphatic invasion (55)	38 (69)	17 (31)	
Lymphatic invasion (49)	29 (59)	20 (41)	NS
Rectum (1734)	1174 (68)	560 (32)	
Age <50 years (226)	157 (69)	69 (31)	
Age 50–75 years (1159)	788 (68)	371 (32)	
Age >75 years (346)	227 (66)	119 (34)	NS
Male (968)	653 (67)	315 (33)	
Female (766)	521 (68)	245 (32)	NS
Dukes' A (197)	135 (68)	62 (32)	
Dukes' B (549)	370 (67)	179 (33)	
Dukes' C (865)	599 (69)	266 (31)	
Dukes' D (113)	64 (57)	49 (43)	NS
Histological grade G1 (213)	148 (69)	65 (31)	
Histological grade G2 (1213)	814 (67)	399 (33)	
Histological grade G3 (227)	162 (71)	65 (29)	NS
Non-mucinous (504)	311 (62)	193 (38)	
Mucinous (38)	29 (76)	9 (24)	NS
No vascular invasion (351)	219 (62)	132 (38)	
Vascular invasion (94)	46 (49)	48 (51)	NS (0.018)
No lymphatic invasion (250)	149 (60)	101 (40)	
Lymphatic invasion (209)	103 (49)	106 (51)	NS (0.027)

Table 2. Prognostic significance for functional categories of *TP53* mutation in site and stage subgroups of colorectal cancer

Tumour site (*n*)	RR	CI (95%)	*P*
All sites (2855)			
Dukes' A			
Active (192)	1.00		
Inactive (72)	1.11	0.69–1.79	NS
Dukes' B			
Active (716)	1.00		
Inactive (274)	0.91	0.72–1.14	NS
Dukes' C			
Active (1012)	1.00		
Inactive (389)	0.91	0.78–1.06	NS
Dukes' D			
Active (121)	1.00		
Inactive (79)	1.71	1.25–2.33	<0.001
Proximal colon (849)			
Dukes' A			
Active (40)	1.00		
Inactive (4)	1.07	0.60–1.65	NS
Dukes' B			
Active (253)	1.00		
Inactive (69)	1.07	0.69–1.65	NS
Dukes' C			
Active (330)	1.00		
Inactive (91)	0.81	0.59–1.10	NS
Dukes' D			
Active (42)	1.00		
Inactive (20)	2.03	1.10–3.73	NS (0.023)
Distal colon (282)			
Dukes' A			
Active (17)	1.00		
Inactive (6)	1.73	0.02–10.4	NS
Dukes' B			
Active (93)	1.00		
Inactive (26)	1.14	0.54–2.39	NS
Dukes' C			
Active (83)	1.00		
Inactive (32)	1.18	0.68–2.06	NS
Dukes' D			
Active (15)	1.00		
Inactive (10)	2.13	0.78–5.79	NS
Rectum (1724)			
Dukes' A			
Active (135)	1.00		
Inactive (62)	1.02	0.59–1.74	NS
Dukes' B			
Active (370)	1.00		
Inactive (179)	0.81	0.61–1.07	NS
Dukes' C			
Active (599)	1.00		
Inactive (266)	0.94	0.78–1.14	NS
Dukes' D			
Active (64)	1.00		
Inactive (49)	1.76	1.16–2.66	0.007

RR, relative risk; CI, confidence interval.

Table 3. Predictive value of functional categories of *TP53* mutation in Dukes' C colorectal cancer

Tumour site (*n*)	RR	CI (95%)	*P*
All sites, active *TP53*			
No chemotherapy (628)	1.00		
Chemotherapy (342)	0.61	0.50–0.73	<0.001
All sites, inactive TP53			
No chemotherapy (236)	1.00		
Chemotherapy (121)	0.63	0.46–0.85	0.003
Proximal colon, active TP53			
No chemotherapy (202)	1.00		
Chemotherapy (111)	0.57	0.41–0.79	<0.001
Proximal colon, inactive TP53			
No chemotherapy (56)	1.00		
Chemotherapy (26)	0.42	0.21–0.85	NS
Distal colon, active TP53			
No chemotherapy (50)	1.00		
Chemotherapy (33)	0.53	0.26–1.09	NS
Distal colon, inactive TP53			
No chemotherapy (19)	1.00		
Chemotherapy (10)	0.91	0.31–2.63	NS
Rectum, active TP53			
No chemotherapy (376)	1.00		
Chemotherapy (198)	0.64	0.50–0.81	<0.001
Rectum, inactive TP53			
No chemotherapy (161)	1.00		
Chemotherapy (85)	0.68	0.47–0.97	NS

functional status for transcriptional ability. The above findings should be interpreted with caution, however, because of the small number of cases in several subgroups and the non-randomised nature of patient selection for chemotherapy.

discussion

Evidence for the heterogeneity of *TP53* mutations with respect to cancer patient outcomes has been available for several years [10–13]. More recent *in vitro* work has quantitated the functional activity of different *TP53* mutations with respect to their ability to transactivate target genes [4]. In the current study we have used knowledge gained from this *in vitro* work to classify *TP53* mutations into active and inactive categories and thereby determine their prognostic and predictive significance in CRC. To do this we have taken advantage of the CRC-TP53 International Collaborative Study database containing information on the *TP53* mutation status from over 3500 CRC patients in 17 countries [7].

Almost one-third of rectal tumours were found to contain inactive *TP53* mutations compared to just 22% for tumours arising in the proximal colon (Table 1). The reasons for this are unknown but may indicate a more important role for *TP53* mutation in the development of rectal compared with proximal tumours. The higher frequency of inactive *TP53* mutations in Dukes' D (39.5%) compared with Dukes' A–C (27.7%) tumours suggests these specific mutations are associated with a more aggressive phenotype. In support of this, tumours with the poor prognosis features of vascular or lymphatic invasion also showed significantly higher frequencies of inactive *TP53* mutations in the overall CRC cohort.

An interesting finding in this study was that inactive *TP53* mutations were a prognostic feature for poor survival only in patients with Dukes' stage D tumours (Table 2). The increased risk of death associated with this functional category of mutation was approximately two-fold. In contrast, no prognostic significance was seen for inactive *TP53* mutations in any of the earlier tumour stage groups. This result cannot be explained by an effect of chemotherapy since only a very small proportion of Dukes' A and B patients received this treatment. Therefore, it appears that not only are inactive *TP53* mutations more frequent in advanced CRC, but any prognostic value is also restricted to late stage tumours.

There was no evidence for a difference in the response of Dukes' C patients to adjuvant chemotherapy according to *TP53* functional status (Table 3). Active and inactive *TP53* groups showed similar levels of benefit from chemotherapy, with a Relative Risk (RR) of 0.61 and 0.63, respectively. Therefore, the functional status of *TP53* mutation for transactivation ability does not appear to influence the response of localised CRC to 5-fluorouracil-based adjuvant chemotherapy. This finding requires confirmation, however, in prospective studies where patients have been randomized to adjuvant treatments. The better survival of adjuvant treated Dukes' C patients in this study may, in part, be explained by selection bias for treatment towards a younger and healthier population.

In view of the relatively high frequency and the prognostic significance of inactive *TP53* mutations in advanced CRC, it will be interesting to determine whether these mutations have predictive value for response to 5-fluorouracil as well as to newer treatments such as oxaliplatin and irinotecan, which act through different cytotoxic mechanisms. The strong and independent prognostic significance reported for *TP53* mutation in breast cancer suggests it will also be interesting to investigate functional categories of mutation in this tumour type, particularly since several studies on large cohorts have already been carried out [14].

This study found that inactive *TP53* mutations had no prognostic significance in Dukes' A–C CRC and no predictive value in Dukes' C CRC. However, the *TP53* mutations were defined by their *in vitro* transactivation ability [4, 5] and it is possible that categorisation according to ability to induce apoptosis would give a different result. Because of the technical challenges involved, the apoptotic ability of different *TP53* mutants remains to be determined and may in any case depend upon the cell type. A recent study found no correlation between the transcriptional activity of 179 mutant *TP53* species and their ability to induce apoptosis of Saos-2 cells *in vitro* [6]. This study also described the existence of 'super *TP53* mutants' with a greatly elevated capacity to induce apoptosis and which cluster at amino acids 121 and 290–292. Unfortunately the small number of such mutants in the present study prevented their investigation for prognostic and predictive significance.

An alternate explanation for the lack of prognostic significance observed for inactive *TP53* mutations in localised CRC is that such changes are important only during the very early stages of carcinogenesis. Because of selective pressures, other genetic and epigenetic changes may later overshadow any

differences in *TP53* functional activity. A further possible explanation relates to the recent discovery of an alternative promoter and of multiple splice variants for *TP53* [15]. At least one of the isoforms, Δ133p53, can have a dominant negative effect on full-length *TP53* and inhibit *TP53*-mediated apoptosis. The differential expression of *TP53* isoforms in human tumours could again overshadow any functional differences between different *TP53* mutants.

The TP53-CRC International Collaborative Study found evidence for prognostic significance of exon 5 mutations in proximal colon tumours and of denaturing mutations in distal colon tumours [7]. That study, however, did not investigate the prognostic significance of different *TP53* mutations in relation to tumour stage. In light of the present finding of strong prognostic value for inactive *TP53* mutations in Dukes' D tumours, it will be interesting to determine whether any of the structural groups of *TP53* mutation also have prognostic significance in advanced CRC.

acknowledgements

Members of the 'TP53-CRC' collaborative group are as follows:
Australia: Barry Iacopetta, Hany Elsaleh, Richie Soong (University of Western Australia, Nedlands).
Austria: Daniela Kandioler, Elisabeth Janschek, Sonja Kappel (University of Vienna Medical School, Vienna).
China: Maria Lung, Cheung-Shing S. Leung, Josephine M. Ko (Dept. of Biology, Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong (SAR), People's Republic of China); Siu T. Yuen, Judy W.C. Ho, S. Y. Leung (Department of Pathology, Queen Mary Hospital, The University of Hong Kong, Pokfulam, Hong Kong).
France: Thierry Soussi (Universite PM Curie, Paris); Evelyne Crapez, Jacqueline Duffour, Marc Ychou (CRLC Val d'Aurelle, Research Cancer Center, Parc Euromédecine, Montpellier)
Ireland: Dermot T. Leahy (School of Medicine and Medical Science, Conway Institute, University College Dublin, Dublin); Diarmuid P. O'Donoghue (Centre for Colorectal Disease, St. Vincent's University Hospital, Dublin).
Italy: Antonio Russo, Viviana Bazan, Valentina Agnese, Sandra Cascio, Gaetana Di Fede, Nicola Gebbia (Department of Oncology, Università di Palermo); Gabriella Dardanoni (Epidemiological Observatory Center of Sicily, Palermo); Luigi Chieco-Bianchi, Roberta Bertorelle (Immunology and Molecular Oncology Unit, Padova City Hospital and Department of Oncology and Surgical Sciences, Oncology Section, University of Padova); Claudio Belluco (Department of Oncology and Surgical Sciences, Surgery Section, University of Padova); Walter Giaretti, Patrizio Castagnola (National Institute for Cancer Research, Dept. Diagnostic Technologies, Lab. Biophysics and Cytometry, Genoa); Enrico Ricevuto, Corrado Ficorella (Medical Oncology Unit, Department of Experimental Medicine, University of L'Aquila, L'Aquila); Silvano Bosari, Carmelo D. Arizzi (Department of Medicine, Surgery and Dentistry, Division of Pathology, University of Milan, AO San Paolo e IRCCS Ospedale Maggiore, Milan).
Japan: Michiko Miyaki (Hereditary Tumor Research Project, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo); Masamitsu Onda (Nippon Medical School, Institute of Gerontology, Department of Molecular Biology, Nakahara-ku, Kawasaki); Shunsuke Kato, Chikashi Ishioka (Department of Clinical Oncology, Institute of Development, Aging, and Cancer, Tohoku University, Sendai).
Netherlands: Ellen Kampman, Brenda Diergaarde (Division of Human Nutrition, Wageningen University, Wageningen).
New Zealand: Janice Royds (Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin).
Norway: Ragnhild A. Lothe, Chieu B. Diep (Department of Genetics, Institute for Cancer Research, the Norwegian Radium Hospital, and Department of Molecular Biosciences, University of Oslo); Gunn I. Meling (Institute of Forensic Medicine, University of Oslo, Rikshospitalet, University Hospital and Department of Surgery, Akershus University Hospital, University of Oslo).
Poland: Jerzy Ostrowski, Lech Trzeciak (Department of Gastroenterology, Medical Center for Postgraduate Education, Maria Sklodowska-Curie Memorial Cancer Center, Warsaw); Katarzyna Guzińska-Ustymowicz, Bogdan Zalewski (Department of General Pathomorphology, Medical University of Białystok).
Spain: Gabriel M. Capellá and Victor Moreno, Department of Epidemiology and Cancer Registry, Institut Català d'Oncologia, L'Hospitalet de Llobregat, Barcelona; Miguel A Peinado, Department of Molecular Oncology, Institut de Recerca, Oncològica, L'Hospitalet de Llobregat, Barcelona.
Sweden: Christina Lönnroth, Kent Lundholm (Göteborg University, Institute of Surgical Sciences, Department of Surgery, Sahlgrenska University Hospital, Göteborg); Xiao-Feng Sun, Agnata Jansson (Department of Oncology, Institute of Biomedicine and Surgery, Linköping University, Linköping).
Switzerland: Hanifa Bouzourene (Institute of Pathology, Centre Hospitalier Universitaire Vaudois, Lausanne).
Taiwan: Ling-Ling Hsieh (Department of Public Health, Chang Gung University, Tao-Yuan); Reiping Tang (Colorectal Section, Chang Gung Memorial Hospital, Tao-Yuan).
Thailand: Duncan R. Smith (Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakorn Pathom).
UK: Timothy G. Allen-Mersh, Zulfiqar AJ Khan (Department of Surgery, Faculty of Medicine, Imperial College of Science Technology and Medicine, Chelsea & Westminster Hospital, London); Andrew J. Shorthouse (Department of Coloproctology, Northern General Hospital, Herries Road, Sheffield).
USA: Mark L. Silverman (Department of Pathology, Lahey Clinic Medical Center, Burlington, MA).

references

1. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000; 408: 307–310.
2. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994; 54: 4855–4878.
3. Soussi T, Beroud C. Assessing *TP53* status in human tumours to evaluate clinical outcome. Nat Rev Cancer 2001; 1: 233–240.

4. Kato S, Han SY, Liu W et al. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. Proc Natl Acad Sci USA 2003; 100: 8424–8429.
5. Soussi T, Kato S, Levy PP, Ishioka C. Reassessment of the *TP53* mutation database in human disease by data mining with a library of *TP53* missense mutations. Hum Mutat 2005; 25: 6–17.
6. Kakudo Y, Shibata H, Otsuka K et al. Lack of correlation between p53-dependent transcriptional activity and the ability to induce apoptosis among 179 mutant p53s. Cancer Res 2005; 65: 2108–2114.
7. Russo A, Bazan V, Iacopetta B et al. The *TP53* Colorectal Cancer International Collaborative Study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment. J Clin Oncol 2005; 23: 7518–7528.
8. Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. Ann Intern Med 1990; 113: 779–788.
9. Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer 2002; 101: 403–408.
10. Borresen AL, Andersen TI, Eyfjord JE et al. *TP53* mutations and breast cancer prognosis: particularly poor survival rates for cases with mutations in the zinc-binding domains. Genes Chromosomes Cancer 1995; 14: 71–75.
11. Berns EM, van Staveren IL, Look MP et al. Mutations in residues of *TP53* that directly contact DNA predict poor outcome in human primary breast cancer. Br J Cancer 1998; 77: 1130–1136.
12. Russo A, Migliavacca M, Zanna I et al. p53 mutations in L3-loop zinc-binding domain, DNA-ploidy, and S phase fraction are independent prognostic indicators in colorectal cancer: a prospective study with a five-year follow-up. Cancer Epidemiol Biomarkers Prev 2002; 11: 1322–1331.
13. Migliavacca M, Ottini L, Bazan V et al. *TP53* in gastric cancer: mutations in the L3 loop and LSH motif DNA-binding domains of *TP53* predict poor outcome. J Cell Physiol 2004; 200: 476–485.
14. Borresen-Dale AL. *TP53* and breast cancer. Hum Mutat 2003; 21: 292–300.
15. Bourdon JC, Fernandes K, Murray-Zmijewski F et al. p53 isoforms can regulate p53 transcriptional activity. Genes Dev 2005; 19: 2122–2137.