ORIGINAL ARTICLE

P53 Expression in Colorectal Carcinoma: The University of Malaya Medical Centre's Experience

K S K Goh, B.Sc. (Hons)*, T A Ong, FRCS, S C Peh, FRCPath*, H L Yuen, B.BioMed (Hons)*, M Naicker, MPath***

*Departments of Pathology, and **Surgery, Faculty of Medicine, Universiti Malaya Medical Centre, 50603 Kuala Lumpur

Summary

Loss of P53 function is regarded as one of the critical steps in colorectal carcinogenesis. This study determines the P53 expression pattern of colorectal carcinoma in a cohort of 116 local patients. There was no significant relationship between overexpression of P53 with tumour stage (p=0.209, chi square test) and grade (p=0.877, chi square test). Survival analysis using Kaplan-Meier procedure did not show significant relationship between P53 positivity with overall recurrence-free and survival outcome (p=0.3322 and 0.921 respectively; log rank test). Long-term follow-up may give a better evaluation on the prognostic value of P53 overexpression in colorectal carcinoma.

Key Words: P53, Colorectal carcinoma, Prognosis, Survival

Introduction

P53, which was originally detected as a 53 kDa nuclear phosphoprotein, is an important regulator of normal cell growth [1, 2]. It arrests the cell from entering S phase at the G1 phase of the cell cycle by activating the expression of P21, a P53 inducible cyclin dependent kinase inhibitor. Such activity prevents the cell from proceeding through the cell cycle [2,3] thereby indirectly suppressing tumour development. In addition, P53 is also an integral component in the pathway of programmed cell death (apoptosis) induced by DNA-damaging factors[3]. However, in tumour cells, the p53 gene is often mutated. The mutant P53 protein losses the normal function of its wild-type counterpart. This loss of function may occur either by genetic mutation in the gene, followed by a deletion of the remaining wild-type allele [4,5] or through inactivation of the wild-type protein after oligomerization with the more stable mutant protein[6]. Inactivated P53 has no growth-suppressive function and this permits the progression of growth-arrested resting cells to an active cycling state. This may result in the immortalisation of normal cells[7]. Also, the mutated P53 protein has been shown to be able to contribute to the transformation of cells in the presence of an activated ras gene[8]. Therefore, this variant of P53 augments the tumorigenic potential of cancer.

P53 protein is constitutively expressed in most normal tissues. The normal cell has a very low quantity of the wild type protein because of it's short half-life of about 6-20 minutes[9]. Thus, it is not detectable by conventional immunoprecipitation or immunohistochemical methods[2]. In contrast, large quantities of P53 (5-100 folds) can be detected in transformed cells in culture and human cancers[2]. Overexpression of P53 has been demonstrated in many solid tumours, including breast,[10] ovarian,[11] endometrial[12], lung[13], and colonic cancers[14]. It is well documented that mutations in the p53 gene represents the most frequent genetic alteration detected in the above malignancies[15]. Majority of the p53 gene mutation are point mutations, which causes amino acid

This article was accepted: 10 April 2004
Corresponding Author: S C Peh, Department of Pathology, Universiti Malaya Medical Center, 50603 Kuala Lumpur

substitution and changes in the conformation of the protein. This results in an increased stability of the protein and a higher steady-state protein level within the cell[6,7,16,17]. The mutant P53 protein is shown to have a prolonged half-life of up to 6 hours[7]. The accumulation of mutant protein may be detected by immunohistochemistry, which has been used in a large number of studies on various tumour types, as a marker for p53 gene mutation or deletion. Various study groups have attributed carcinogenesis of colorectal carcinoma to p53 gene mutation and its overexpression[17-22].

Here, we undertook a study to determine the pattern of P53 overexpression in colorectal carcinoma in a cohort of Malaysian patients, and evaluate the practical prognostic value of immunohistochemistry analysis of P53 overexpression with a clinical follow-up.

Materials and Methods

One hundred and sixteen consecutive cases of primary colorectal carcinoma diagnosed for the first time between January 1999 and December 2000 were retrieved from the archives for the study. The original slides were reviewed for confirmation of diagnosis, then subtyped into mucinous and conventional adenocarcinoma. The latter was graded according to tumour histological differentiation (well, moderate and poor). The tumours were also staged using Dukes' classification, and with distant metastasis designated as stage D. The presentation of tumours were divided into 2 major sites, the right colon which includes the caecum to the transverse colon and the left colon, which includes the splenic-flexure to the rectum. In cases with lymph node metastasis at presentation, the lymph node tissue block containing the highest number of tumour metastasis was selected for P53 staining, together with the primary tumour. Information pertaining to the demographic data, clinical presentation and progress were obtained from patients' records. Data on the age at presentation, sex, ethnic group, tumour site of presentation, clinical appearance of primary tumour (polyp, ulceration, stenosis) and disease progression was compiled. Recurrence-free period was defined as the time (months) from the date of diagnosis to the date of clinically confirmed recurrence, whereas overall survival period was defined as the time (months) from the date of diagnosis to the date of death.

Immunohistochemical Staining

Serial 4μm sections from formalin fixed and paraffin embedded tissue blocks were used for further immunohistochemical staining and evaluation. Antigen was retrieved by heating for 20 minutes in a microwave oven at 99°C, and incubated with the primary antibody, DO-7 (Dako®, Golstrup, Denmark). A secondary peroxidase conjugated goat anti-mouse antibody (Dako EnVision+™, Carpinteria, California, USA) was applied. The immunohistochemical reactions were developed in freshly prepared 3,3-diaminobenzidine (Dako® Liquid DAB+ Substrate-Chromogen System, Carpinteria, California, USA). Control staining was carried out by using sections of squamous cell carcinoma and normal colonic mucosa, both for positive and negative control, respectively.

Evaluation of P53 overexpression

The slides were examined and scored independently by 2 pathologists (PSC, MN) without any clinical or pathological information. The intensity of the P53 nuclear staining on neoplastic cells was scored as follows: uniformly very strong (+++); strong but variable (++), weak (+) and negative (-). The percentage of P53-immunopositive cancer cells was evaluated on at least 3 different fields on at least 1,000 tumour cells. Only those with strong (≥10%) positive staining were categorised as overexpression of P53 protein.

Statistical Analysis

Differences between categorical variables were calculated using the chi-square test, while recurrence-free and overall survival analysis was performed using Kaplan-Meier test. Log-rank test was used to compare the different survival curves. P value of less than 0.05 was chosen for interpretation of statistical significance. Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences) software, version 10.0.

Results

There were 56 male and 60 female patients. Their ages range from 28 to 88 years with a mean of 63.25 years (SD:11.41 years). In this cohort of patients, 19 (16.4%) were Malays, 84 (72.4%) Chinese and 13 (11.2%) Indians. The average follow-up period was 15.2 months (SD:8.4 months) and a median of 15 months. According to histological grading, there were 15 (12.9%) well-differentiated, 80 (69%) moderately-

differentiated and 12 (10.3%) poorly differentiated tumours of conventional type. The remaining 9 (7.8%) were mucinous tumours (Table I). According to Dukes' staging system, 15 (12.9%) cases were in stage A, 54 (46.6%) cases in stage B, 39 (33.6%) cases in stage C and 8 (6.9%) cases in stage D. 19 (16.4%) tumours were located in the right colon and 97 (83.6%) in the left colon.

P53 immunoreactivity was detected as nuclear staining in the neoplastic cells. Staining in the nuclei was granular or reticular. There was no reactivity observed in normal colonic mucosa adjacent to the carcinoma, or in stromal and smooth muscle cells. Pale staining of endothelial cells and connective tissue, which was clearly different from the nuclear staining of neoplastic cells, was considered as background staining and not included in the evaluation. Negative control tissue sections did not show any staining. Based on the intensity of the immunohistochemical reaction and the percentage of stained nuclei, three patterns of P53 immunoreactivity could be discerned in the colorectal adenocarcinomas examined: Type 1: no detectable nuclear staining; Type 2: nuclear staining generally of weak intensity and of a low percentage of tumour cells (<10%) unevenly distributed; Type 3: strong nuclear staining of a large number (from 10% to nearly 100%) of neoplastic cells. Tumours with staining patterns Type 1 and Type 2 were regarded as p53 negative. It was observed that 79 of 116 (68.1%) cases showed Type 3 p53 positive immunoreactivity (Table I).

The incidence of P53 overexpression was 66.7% (10/15) in Dukes' A, 68.5% (37/54) in Dukes B, 61.5% (24/39) in Dukes' C and 100% (8/8) in stage D, whereas it was 66.7% (10/15) in well-differentiated tumours, 70.0% (56/80) in moderately differentiated and 58.3% (7/12) in poorly differentiated tumours. For the mucinous type, 66.7% (6/9) cases showed strong P53 overexpression. Statistical analysis showed there was no definite association between P53 overexpression with tumour stage and grade (P=0.209 and 0.877 respectively, chi square test). There was also no significant association of P53 overexpression with lymph node metastasis (Dukes' C) at presentation (p=1.00, chi square test). The frequency of P53 overexpression in the metastasised cases (Dukes' C and stage D) appears higher when compared to localised cases (Dukes' A and B), being 68.1% and 66.7% respectively (Table I). However, this difference is not statistically significant (p= 1.00, chi square test).

The P53 immunoreactivity of the metastasised tumours were similar to the primary lesions, in that, the 31 cases of primary tumours with P53-positive staining, the metastasised tumours also showed positive staining with similar quantum and intensity. Likewise, in the 15 cases of P53-negative primary tumours with metastasis, the corresponding metastasised tumours showed negative staining. The tumours from Indian patients showed significantly less P53 positivity when compared to the non-Indian group (38.5% vs 75%) (P=0.033, chi square test). However, there was no predilection of P53 nuclear overexpression for gender (P=0.503, chi square test), the clinical appearance of primary tumour (polyp, ulcerative, stenosis) (P=0.741, chi square test) and the site of tumour presentation (P=0.813, chi square test).

In the study of clinical outcomes, 35 and 38 cases were excluded in the local and systemic recurrence-free analysis respectively due to incomplete data. During a median follow-up of 15.00 months (SD: 8.42 months), 23 patients (19%) had recurrence of the cancer, 8 developed local recurrence and the other 15 had systemic recurrence.

Four of 57 (7.0%) P53 positive tumours had local recurrence when compared to the 4 of 23 (17.4%) P53-negative tumours. Kaplan-Meier survival analysis showed there was no significant difference in the time interval of onset of recurrence between the two groups (28.86 vs 26.34 months, P=0.1754, log-rank test) (Figure 1). 10 of 56 (17.9%) p53-positive tumours had systemic recurrence when compared to 5 of 22 (22.7%) P53-negative tumours. The mean interval of P53-positive tumours to recur was 26.31 months, whereas the P53-negative group was 24.96 months. There was also no significant difference between P53-positive and negative tumours in the time interval for a systemic recurrence to occur (P=0.6655, log rank test) (Figure 2).

Thirty four cases were excluded in the overall survival study, due also to incomplete data. The study of overall survival showed 4 of 56 patients (7.3%) with P53-positive tumour died, whereas 2 of 26 patients (7.7%) with P53-negative tumour died. There was no difference in the mean survival time of P53 negative and positive tumours (Figure 3, p=0.921, log rank test). The results indicate that P53 overexpression does not influence the risk of tumour recurrence and survival of patients.

Table I: Characteristics of patients

		No. of cases (%)	P53 positive (%)
Stage	Duke A	15 (12.9)	10 (66.7)
	Duke B	54 (46.6)	37 (68.5)
	Duke C	39 (33.6)	24 (61.5)
	Stage D	8 (6.9)	8 (100)
	Total	116	79 (68.1)
Histologic Grade	Well differentiated	15 (12.9)	10 (66.7)
	Moderately Differentiated	80 (69.0)	56 (70.0)
	Poorly Differentiated	12 (10.3)	7 (58.3)
	Mucinous	9 (7.8)	6 (66.7)
	Total	116	79 (68.1)
Race	Malay	19 (16.4)	15 (78.9)
	Chinese	84 (72.4)	59 (70.2)
	Indian	13 (11.2)	5 (38.5)
	Total	116	79 (68.1)

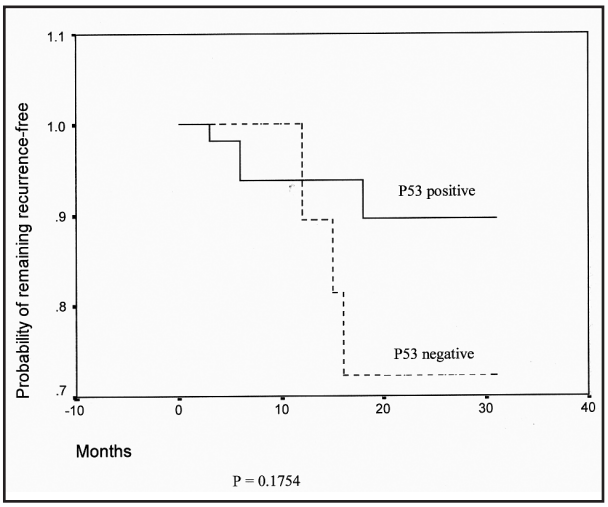

Fig. 1: Kaplan-Meier survival curves of Recurrence-free survival (local recurrences) and p53 status

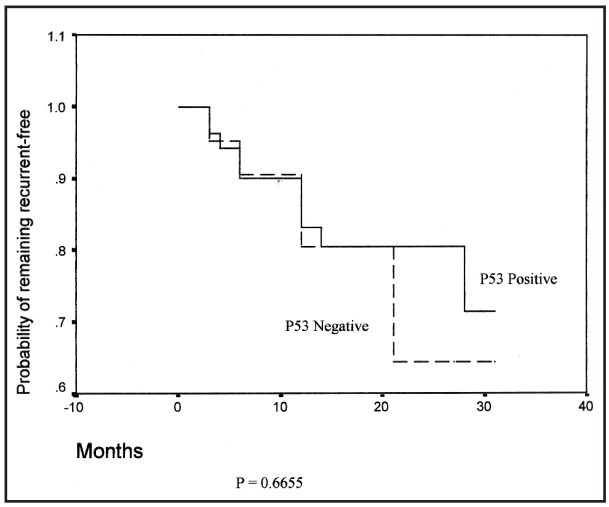

Fig. 2: Kaplan-Meir survival curves of Recurrence-Free survival (systemic recurrences) and p53 status

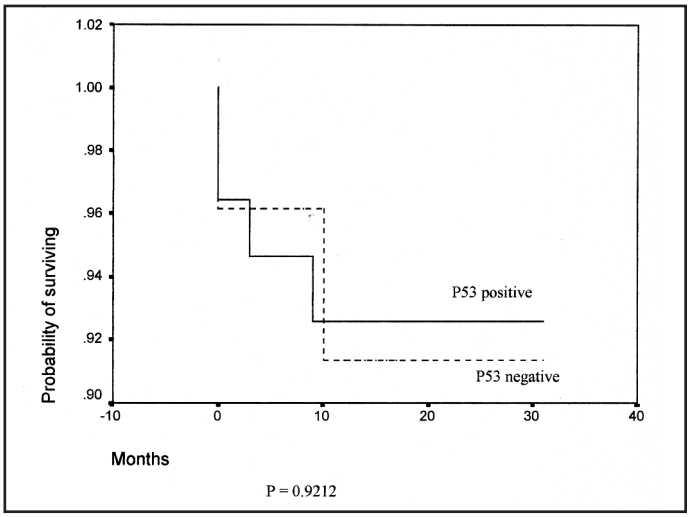

Fig. 3: Kaplan-Meier survival curves of overall survival and p53 status

Discussion

Overexpression of P53 protein had been well demonstrated in colorectal carcinomas by several study groups [16,20,23,26,27,30-34] and the incidence ranges from 40% to 70% of the total sample. In our study, P53 overexpression was demonstrated in 68.1% of Malaysian colorectal carcinoma cases. Majority of the carcinoma cases are expected to overexpress P53 protein because the gene mutation which gives the mutant protein its stability, are late events in colorectal tumorigenesis. This is supported in the studies by Baker et al [5] and Campo et al [30], where p53 gene mutations were rarely found in adenomas but were frequent in carcinomas. Most of the mutated p53 gene in the carcinomas also had a deletion in the allele, where one normal copy of the gene is deleted. With the loss of the normal gene in the allele, the effects of the mutated gene could not be masked. Studies have also shown a strong correlation between P53 overexpression with gene mutations and allelic deletions[5,16,19,30,34]. The clinical significance P53 expression in colorectal carcinoma is presently still controversial. Some studies could not find a significant association of P53 overexpression with tumour grade, stage, DNA ploidy, proliferation rate and sex of patients[5,19,23,26,30,32-34]. On the other hand, a few studies found some clinical correlation mentioned above[20,27,29,31].

We found no significant association of P53 overexpression with stage (Dukes' stage) and grade of tumours in our series. However, the incidence of P53 overexpression seems higher in stage D when compared to that in Dukes' stage A, B and C although not statistically significant (P=0.107). Kawasaki et al [20] also noted that the difference in P53 overexpression was significant with Dukes' A, B, and C as one group when compared with stage D.

Tomoda et al [29] has observed that most gastric and colorectal tumours with P53 overexpression usually have a high proliferative activity and a tendency to metastasise to lymph nodes. They suggested that the failure of the overexpressed P53 protein in regulating cell growth causes active cell proliferation, which then increases the risk of lymph node metastasis[29,36,37]. However, our study did not demonstrate this phenomenon, hence concurring with the observations of Purdie et al[32]. The lack of association of P53 overexpression with Dukes' stage probably indicate that P53 expression is not important in driving the tumour towards lymph node metastasis.

A notable finding in our study was a significant difference in P53 overexpression in the tumours from Indian patients when compared to non-Indian patients. However, the number of cases from Indian patient group was relatively small (13) and definitive

conclusion awaits in the study of bigger series. The incidence of P53 overexpression in the mucinous grade was also higher in our study, which is in contrast to other reports, where the incidence is always lower[19,28].

Despite many studies reporting the association of p53 gene mutations with poor prognosis and shorter survival in colorectal carcinoma patients, controversial results still prevail. P53 overexpression had been found to adversely affect prognosis by some authors[25,27,33], but this observation had not been confirmed by others[14,23,26,29]. Our results from this study concur with the latter. There are many proposed explanations regarding the controversial results. Yamaguci et al[33] attributed the difference to the type of antibody used, because he found a difference in the survival using Pb1801 antibody, compared to Pb421, used by another group[26]. Scott et al[26] reasoned that the lack of correlation with established prognostic indicators such as stage, grade, and DNA ploidy is consistent with the failure of P53 expression to predict survival.

In conclusion, our study showed that P53 overexpression has no significant correlation with the clinical stage of the tumour at the time of presentation and tumour grade. Its prognostic predictive role remained to be ascertained.

Conclusion

The data from this study does not support the association and prognostic value of P53 overexpression with colorectal carcinoma. Larger studies with a longer follow-up time could give more conclusive information.

Acknowledgements

This study is supported by the Top-Down Biotechnology grant 26-02-03-0586 given by the Ministry of Science and Technology, Malaysia. We would also like to thank Ms. Karina Razali for her assistance in the statistical calculations.

References

1. Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. Nature 1979; 278: 261-63.
2. Chang F, Syrjanen S, Kurvinen K, Syrjanen K. The p53 tumor suppressor gene as a common cellular target in human carcinogenesis. Am J Gastroenterol 1993; 88(2): 174-86.
3. el-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. Cell 1993; 75(4): 817-25.
4. Harris CC. P53: at the crossroad of molecular carcinogenesis and risk assessment. Science 1993; 262: 1980-81.
5. Baker SJ, Preisinger AC, Jessup JM, et al. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res 1990; 50(23): 7717-22.
6. Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. Activating mutations for transformation by p53 produce a gene product that forms an hsc700-p53 complex with an altered half-life. Mol Cell Biol 1988; 8: 531-39.
7. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature 1991; 351: 453-6.
8. Parada LF, Land H, Weineberg RA, Wolf D, Rotter V. Coorperation between gene encoding p53 tumour antigen and ras in cellular transformation. Nature 1984; 312: 649-51.
9. Milner J, Medcalf EA, Cook AC. Tumour suppressor p53: analysis of wild-type and mutant complexes. Mol Cell Biol 1991; 11: 12-19.
10. Proser J, Thompson AM, Cranston G, Evans HJ. Evidence that p53 behaves as a tumour suppressor gene in sporadic breast tumours. Oncogene 1990; 5: 1573.

11. Marks JR, Davidoff Am, Kerns BJ, et al. Overexpression and mutation of p53 in epithelial ovarian cancer. Cancer Res 1991; 51: 2979.

12. Kohler MF, Berchuk A, Davidoff AM, et al. Overexpression and mutation of p53 in endometrial carcinoma. Cancer Res 1992; 52: 1622.

13. Bartek J, Bartkova J, Vojtesek B, et al. Aberrant expression of the p53 oncoprotein is a common feature of wide spectrum of human malignancies. Oncogene 1991; 6: 1699-703.

14. Lanz G, Maestri I, Dubini A, et al. p53 expression in colorectal cancer: Relation to tumour type, DNA ploidy pattern, and short-term survival. Am J Clin Pathol 1996; 105(5): 604-12.

15. Nigro JM, Baker SJ, Preisinger AC, et al. Mutations in the p53 gene occur in diverse human tumour types. Nature 1989; 342: 705-708.

16. Dix B, Robbins P, Carello S, House A, Iacopetta B. Comparison of p53 gene mutation and protein overexpression in colorectal carcinomas. Br J Cancer 1994; 70: 585-90.

17. Cripps KJ, Purdie CA, Carder PJ, et al. A study of stabilization of p53 protein versus point mutation in colorectal carcinoma. Oncogene 1994; 9: 2739-43.

18. Tan TH, Wallis J, Levine AJ. Identification of the p53 protein domain involved in the formation of the SV40 larger T antigen p53 protein complex. J Virol 1986; 59: 574-83.

19. Costa A, Marasca R, Valentinis B, et al. p53 gene point mutations in relation to p53 nuclear protein accumulation in colorectal cancers. J Pathol 1995; 176: 45-53.

20. Kawasaki Y, Monden T, Morimoto H, et al. Immunohistochemical study of p53 expression in microwave-fixed, paraffin-embedded sections of colorectal carcinoma and adenoma. Am J Clin Pathol 1992; 97: 244-49.

21. Fearon ER, Volgestein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61: 759-67.

22. Kikunchi-Yanoshita R, Konishi M, Ito S, et al. Genetic changes of both p53 alleles associated with the conversion from colorectal adenoma to early carcinoma in familial adenomatous polyposis and non-familial adenomatous polyposis patients. Cancer Res 1992; 52: 3965-71.

23. Kressner ULF, Lindmark G, Gerdin B, Pahlman L, Glimelius B. Immunohistochemical p53 staining is of limited value in the staging and prognostic prediction of colorectal cancer. Anticancer Res 1996; 16: 951-58.

24. Wynford-Thomas D. p53 in tumour pathology. Can we trust immunocytochemistry? J Pathol 1994; 166: 329-30.

25. Remnikos Y, Tominaga O, Hammel P, et al. Increased p53 protein content of colorectal tumours correlates with poor survival. Br J Cancer 1992; 66: 758-64.

26. Scott N, Sagar P, Stewart J, Blair GE, Dixon MF, Quirke P. p53 in colorectal cancer: clinicopathological correlation and prognostic significance. Br J Cancer 1991; 63: 317-9.

27. Starzynska T, Bromley M, Ghosh A, Stern PL. Prognostic significance of p53 overexpression in gastric and colorectal carcinoma. Br J Cancer 1992; 66: 558-62.

28. Hanski C, Bornhoeft G, Shimoda T, et al. Expression of p53 protein in invasive colorectal carcinoma of different histologic types. Cancer Res 1992; 70: 2772-77.

29. Tomoda H, Kakeji Y. Immunohistochemical analysis of p53 in colorectal cancer regarding clinicopathological correlation and prognostic significance. J Surg Oncol 1995; 58: 125-28.

30. Campo E, de la Calle-Martin O, Miquel R, et al. Loss of heterozygosity of p53 gene and p53 protein expression in human colorectal carcinogensis. Cancer Res 1991; 51: 4436-42.

31. Auvinen A, Isola J, Visakorpi T, et al. Overexpression of p53 and long-term survival in colon carcinoma. Br J Cancer 1994; 70: 293-6.

32. Purdie CA, Grady JO, Piris J, Wylie AH, Bird CC. P53 expression in colorectal tumours. Am J Pathol 1991; 138(4): 807-13.

33. Yamaguchi A, Kurosaka Y, Fushida S, et al. Expression of p53 protein in colorectal cancer and its relationship to short-term prognosis. Cancer 1992; 70(12): 2778-84.

34. Cunningham J, Lust JA, Schaid DJ, et al. Expression of p53 and 17p allelic loss in colorectal carcinoma. Cancer Res 1992; 52: 1974-80.

35. Dunn JM, Hastrich DJ, Newcomb P, et al. Correlation between p53 mutations and antibody staining in breast carcinoma. Br J Surg 1993; 80: 1410-412.

36. Kakeji Y, Korenaga D, Tsujitani S, et al. Predictive value of Ki-67 and argyrophilic nucleolar organizer region staining for lymph node metastasis in gastric cancer. Cancer Res 1991; 51: 3503-506.

37. Kakeji Y, Korenaga D, Tsujitani S, et al. Gastric cancer with p53 overexpression has high potential for metastasising to lymph nodes. Br J Cancer 1993; 67: 589-93.