Prognostic value of p53 protein expression for patients with gastric cancer – a multivariate analysis

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Summary Mutations in the p53 gene, one of the most common genetic alterations in human cancer, are implicated in tumorigenesis and tumour progression. Although p53 protein expression appears to be correlated to prognosis in patients with malignancy, its prognostic role in gastric cancer has remained controversial. We examined the clinical significance of p53 overexpression in 427 patients with gastric cancer, using multivariate analysis. Tumour sections of gastric cancer tissues from these 427 Japanese patients were stained immunohistochemically with monoclonal antibody PAb1801. The presence of p53 expression was statistically compared with clinicopathological features and post-operative survival, using univariate and multivariate analyses. p53 expression was detected in 38.6% (165 out of 427) of these gastric cancers and immunoreactivity was not observed in normal mucosa adjacent to the tumour. A higher rate of p53 detection was observed among large tumours and in those with a prominent depth of invasion, lymphatic and vascular invasion and lymph node involvement. Prognosis was significantly worse for patients with p53-positive-staining tumours. The 5-year survival rate was 62.5% for patients with p53-negative tumours and 43.3% for those with positive malignancies. p53 expression was a significant prognostic factor for node-positive gastric cancers in subjects undergoing treatment with curative resection, as assessed by Cox regression analysis. Thus, the expression of p53 was closely related to the potential for tumour advance and a poorer post-operative prognosis for patients with gastric cancer.

Keywords: gastric cancer; p53 expression; prognosis; multivariate analysis

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of the number of sites on the organs, when there was no evidence of incurable factors such as peritoneal dissemination, liver metastasis and widespread nodal involvement (Korenaga et al., 1988). The lymph nodes in groups 1, 2 and 3 are referred to as n1, n2 and n3, respectively, based on lymph node metastasis. Lymph node dissection was classified as follows: D1, complete removal of group 1 lymph node alone; D2, complete removal of group 1 and 2 lymph nodes; and D3, complete removal of group 1, 2 and 3 lymph nodes. The pathological diagnosis and classification was according to the General Rules for the Gastric Cancer Study in Surgery and Pathology in Japan (Japanese Research Society for Gastric Cancer, 1981, 1993). Curability A means no residual tumours with a high probability of cure, under the following conditions: no serosal invasion; n0 treated by D1, 2, 3 or n1 treated by D2 or 3; MO, peritoneal dissemination negative (P0), liver metastasis negative (H0); and the proximal and distal margins > 10 mm. Curability B means no residual tumours but not evaluable as ‘curability A’, and curability C shows definite residual tumour. Tumour-advance stage was determined by the TNM classification of UICC (Sobin and Wittekind, 1997).

Three patients (0.07%) died within the first 30 post-operative days. Of the remaining 424 patients, 173 are alive at the time of writing this report. Recurrence of the gastric cancer and death occurred in 173 patients, 55 died of another disease and 13 died of undetermined causes. Death owing to causes other than gastric cancer were considered as censored data in the statistical analysis.

**p53 staining**

Tissue sections were immunostained with a monoclonal antibody against p53 (PAb1801, Oncogene Science, USA) (Banks et al, 1986; Porter et al, 1992; Sarbia et al, 1994; Oiwa et al, 1995). This antibody recognizes a denaturation-resistant epitope between amino acids 32 and 79 of both wild-type and mutant conformations. Xylene was used to remove paraffin from the 5-μm sections, then the sections were progressively hydrated in decreasing concentrations of ethanol. The slides were placed in a thermostatic beaker filled with 0.1 m phosphate-buffered saline (PBS) (pH 7.4) and autoclaved at 121°C to allow the fixed embedded tissue antigen to react with the monoclonal antibody. The sections were then cooled down to room temperature for about 20 min and rinsed in PBS. These sections were then covered with normal rabbit serum for 15 min to reduce non-specific staining, then incubated with a 1:100 dilution of primary antibody at room temperature for 1 h. Next, the sections were washed with PBS, incubated with a 1:600 dilution of biotinylated goat anti-mouse IgG (Dako, Denmark) at room temperature for 30 min and then covered with a 1:1000 dilution of labelled streptavidin peroxidase (Dako) at room temperature for 30 min. The antibody was localized with 3,3'-diaminobenzidine tetrahydrochloride and 0.065% sodium azide was used to block endogenous peroxidase.

We stained both the deep periphery of the tumour and adjacent tumour-free tissue. A distinct nuclear immunoreaction for p53 was recorded as positive, and here the nuclear staining pattern was diffuse with little variation. When 10% of the cancer cells showed a positive nuclear staining, a positive-staining was defined (Kakeji et al, 1993).

**Post-operative chemotherapy**

All of the patients with advanced stage of gastric cancer (T2–4) were treated with post-operative chemotherapy (Sugimachi et al, 1997). An intravenous injection of 10 mg mitomycin C (Kyowa Hakko, Japan) was given on the day of operation, and fluorinated pyrimidine UFT (Taiho Pharmaceutical, Japan) orally in a daily dose of 400 mg was started 2 weeks after the operation and was continued for 1 year.

**Statistical analysis**

The BMDP Statistical Package program (BMDP; Los Angeles, CA, USA) for the IBM (Armonk, NY, USA) 3090 mainframe computer was used for all analyses (Dixon, 1988). The BMDP P4F and P3S programs were used for the chi-squared test and the Mann–Whitney test to compare data from patients with p53-negative and p53-positive tumours. The BMDP P1L program was used to analyse survival rates by the Kaplan–Meier method, and the Mantel–Cox method to test for equality of the survival curves. The BMDP P2L program was used for simultaneous multivariate adjustment of all covariates by the Cox regression analysis with the forward stepwise model (Cox, 1972). The level of significance was $P < 0.05$.

**RESULTS**

The monoclonal antibody pAb1801 has been widely used for p53 protein expression in various cancer tissues. The positive rate of p53 expression in gastric cancer cells was 36.8% (165 out of 427). The staining of p53 was nuclear and p53 was never observed in the normal gastric mucosa examined in all 427 cases, as shown in Figure 1.

**Clinicopathological factors**

Table 1 shows clinicopathological data on 262 patients in whom there was no p53 expression and for 165 patients with p53 overexpression. In the p53-positive patients, the entire stomach was more likely to be involved, and the tumour was larger compared with the p53-negative cases. There were no differences in tissue differentiation and growth patterns. Serosal invasion was more prominent, lymphatic and vascular involvement was more common and the rate of lymph node metastasis was higher in p53-positive tumours.

Surgical management was also compared between the groups (Table 2). There was no difference with regard to the extent of lymph node dissection. Because the entire stomach was more commonly involved, the rate of total gastrectomy was higher in the p53-positive patients. The rate of operative curability C (non-curative resection) was higher in p53-positive patients because of a more advanced stage of the tumour.

**Survival rates**

Post-operative survival curves for patients with p53-negative and p53-positive tumours are shown in Figure 2. The non-gastric cancer deaths were considered as lost to follow-up, as of time of death, and data on survivors with a follow-up time shorter than 10 years were censored from analysis. The 5-year survival rate was 62.5% for p53-negative patients and 43.3% for p53-positive patients ($P < 0.01$). Next, we determined survival rates for patients with p53-negative and p53-positive tumours for tumour size, serosal invasion,
lymphatic and vascular invasion, lymph node metastasis and operative curability, which were identified as factors having a close relation with p53 expression as shown in Tables 1 and 2. In the subgroups of tumour size over 5 cm, serosal invasion positive, lymphatic and vascular invasion positive and lymph node metastasis-positive tumours, and no residual tumours by curative resection (curability A and B), a significantly shorter survival time was noted in p53-positive patients compared with p53-negative patients (Table 3). Survival curves for patients with p53-negative or p53-positive gastric cancers were determined for each node-negative and node-positive group (Figure 3A and B).

Recurrence pattern in curatively resected patients

Data from patients with p53-positive tumours and p53-negative tumours who underwent curative resection (curability A and B) were analysed for recurrence patterns (Table 4). There was a recurrence in 39 out of 204 cases (19.1%) of p53-negative patients and in 29 out of 106 (27.4%) of p53-positive patients. In both groups, recurrence was noted in the peritoneum, distant organs and lymph nodes.

Multivariate analysis of p53 protein expression

Data from Cox regression analysis of all factors are listed in Table 1, and revealed that lymph node metastasis, serosal invasion, liver metastasis, tumour size and peritoneal dissemination proved to be

| Variable                          | p53 negative (n = 262) | p53 positive (n = 165) | P-value |
|-----------------------------------|------------------------|------------------------|---------|
| Age (years)                       | 61.6 ± 12.5*           | 62.1 ± 12.6            | 0.6871  |
| Sex                               |                        |                        |         |
| Men                               | 175 (66.8)             | 120 (72.7)             | 0.1964  |
| Women                             | 87 (33.2)              | 45 (27.3)              |         |
| Tumour maximal diameter (cm)      | 5.89 ± 3.87*           | 7.33 ± 4.19            | 0.0002  |
| Location of tumour                |                        |                        |         |
| Upper                             | 57 (21.8)              | 43 (26.0)              | 0.0285  |
| Middle                            | 84 (32.1)              | 35 (21.2)              |         |
| Lower                             | 95 (36.2)              | 59 (35.8)              |         |
| Whole stomach                     | 26 (9.9)               | 28 (17.0)              |         |
| Histology                         |                        |                        |         |
| Differentiated                    | 123 (47.3)             | 67 (40.6)              | 0.1757  |
| Undifferentiated                  | 137 (52.7)             | 98 (59.4)              |         |
| Specific                           | 2                      | 0                      |         |
| Serosal invasion                  |                        |                        |         |
| Negative (t1,t2)                  | 149 (56.9)             | 74 (44.8)              | 0.0155  |
| Positive (t3,t4)                  | 113 (43.1)             | 91 (55.2)              |         |
| Histological growth pattern       |                        |                        |         |
| Expansive                         | 45 (20.9)              | 25 (16.7)              | 0.4977  |
| Intermediate                      | 67 (31.2)              | 45 (30.0)              |         |
| Infiltrative                      | 103 (47.9)             | 80 (53.3)              |         |
| Unknown                           | 47                     | 15                     |         |
| Lymphatic involvement             |                        |                        |         |
| Negative                          | 138 (52.9)             | 65 (39.6)              | 0.0078  |
| Positive                           | 123 (47.1)             | 99 (60.4)              |         |
| Unknown                           | 1                      | 1                      |         |
| Vascular involvement              |                        |                        |         |
| Negative                           | 205 (78.5)             | 108 (67.1)             | 0.0080  |
| Positive                           | 56 (21.5)              | 53 (32.9)              |         |
| Unknown                           | 1                      | 4                      |         |
| Histological lymph node metastasis|                        |                        |         |
| Negative                          | 120 (46.5)             | 49 (29.9)              | 0.0007  |
| Positive                           | 138 (53.5)             | 115 (70.1)             |         |
| Unknown                           | 4                      | 1                      |         |
| Peritoneal dissemination           |                        |                        |         |
| Negative                          | 246 (93.9)             | 151 (91.5)             | 0.3492  |
| Positive                           | 16 (6.1)               | 14 (8.5)               |         |
| Liver metastasis                  |                        |                        |         |
| Negative                          | 255 (97.3)             | 156 (94.5)             | 0.1404  |
| Positive                           | 7 (2.7)                | 9 (5.5)                |         |
| Stage (UICC)                      |                        |                        |         |
| Ia                                 | 80 (30.5)              | 28 (17.0)              | 0.0089  |
| Ib                                 | 25 (9.5)               | 12 (7.3)               |         |
| II                                 | 39 (14.9)              | 29 (17.6)              |         |
| IIIa                               | 51 (19.5)              | 30 (18.2)              |         |
| IIib                               | 25 (9.5)               | 23 (13.9)              |         |
| IV                                 | 42 (16.1)              | 43 (26.0)              |         |

NS, no significant difference; figures in parentheses are percentages; *mean ± standard deviation; ‡ unknown and specific cases were excluded from statistical analysis.
Curability

Lymph node dissection

the patients with p53-positive tumours (gastric cancers. When the deaths were considered as gastric cancer-related, the patients with p53-positive tumours (n = 165) had a shorter survival time than did those with p53-negative tumours (n = 262) (P = 0.0002). Solid line, p53-negative patients; light line, p53-positive patients

Table 2 Surgical management of patients with p53-negative or p53-positive gastric cancer

| Variable                  | p53 negative (n = 262) | p53 positive (n = 165) | P-value |
|---------------------------|------------------------|------------------------|---------|
| Operative procedure       |                         |                        |         |
| Partial                   | 151 (57.9)             | 68 (41.7)              | 0.0012  |
| Total                     | 110 (42.1)             | 95 (58.3)              |         |
| Unknown*                  | 1                      | 2                      |         |
| Lymph node dissection     |                         |                        |         |
| D0 and D1                 | 60 (22.9)              | 51 (30.9)              | 0.0662  |
| D2 and D3                 | 202 (77.1)             | 114 (69.1)             |         |
| Curability                |                         |                        |         |
| Curability A, B           | 204 (78.5)             | 106 (64.2)             | 0.0030  |
| Curability C              | 56 (21.5)              | 59 (35.8)              |         |

NS, no significant difference; figures in parentheses are percentages; *unknown cases were excluded from statistical analysis.

Figure 2 Survival curves for patients with p53-negative or p53-positive gastric cancers. When the deaths were considered as gastric cancer-related, the patients with p53-positive tumours (n = 165) had a shorter survival time than did those with p53-negative tumours (n = 262) (P = 0.0002). Solid line, p53-negative patients; light line, p53-positive patients

Figure 3 Survival curves for patients with p53-negative or p53-positive gastric cancers in each node-negative and node-positive group. (A) There was no difference between p53-negative (n = 120) and p53-positive patients (n = 49) for the node-negative group (P = 0.9583). (B) Survival time for p53-positive patients (n = 115) was shorter than for p53-negative patients (n = 138) in those in the node-positive group (P = 0.0060). Solid line, p53-negative patients; light line, p53-positive patients

common event in gastric cancer, occurring from the early stage of progression with its specific mutation spectrum (Kim et al, 1991; Yokozaki et al, 1992; Uchino et al, 1993). The mutated p53 gene loses function as a tumour suppressor and can act as a dominant oncogene (Levine et al, 1991). Loss of p53 function accelerates the process of tumorigenesis and alters the phenotype of cancer cells and the response of cells to agents that damage DNA (Carson and Lois, 1995; Chang et al, 1995). Mutant-type p53 proteins have a prolonged half-life, and are thus more likely than the wild-type protein to be detected using immunohistochemical assays (Finlay et al, 1988; Gannon et al, 1990; Levine et al, 1991).

The antibody PAb1801, which we used, recognizes both the wild-type and mutant forms of p53 (Banks et al, 1986; Porter et al, 1992). No reactivity was observed in any normal gastric mucosa adjacent to the tumour tissue and the normal protein has a very short half-life, suggesting that the immunoreactivity of p53 in the tumour tissue itself is likely to represent mutant forms of p53 and relates to a more malignant biological character. Conversely, 61.4% of the gastric cancers in this study were p53 negative. The tumours with immunohistochemically undetectable p53 could represent a decreased availability of the epitope of p53 protein as a result of fixation in paraffin, tumours with normal levels of wild-type p53, tumours with both alleles of the p53 gene deleted, or tumours expressing a mutant p53 protein not identified by the antibody used in this study (Gabbert et al, 1995).

DISCUSSION

In this study, we made use of immunohistochemical staining to examine the relationship between p53 expression, clinicopathological factors and survival, in tissue samples from 427 patients resected because of gastric cancer.

Alterations in the tumour-suppressor p53 gene play a role in the genesis of diverse types of human malignancies (Harris and Hollstein, 1993; Soussi et al, 1994; Chang et al, 1995), and allelic losses and point mutations in the counterpart of the locus of the p53 gene in chromosome 17 are frequent. p53 mutation is a

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The p53 abnormal staining was reported to be related to the proliferating activity of cancer cells (Kakeji et al, 1993), and aggressive behaviour of cancer cells for serosal invasion, lymph node metastasis of gastric cancer and a poorer prognosis ensued (Martin et al, 1992; Starzynska et al, 1992; Joypaul et al, 1994; Maehara et al, 1995; Mönig et al, 1997). Because vascular and lymphatic involvement are closely related to p53 expression, p53 expression was closely related to tumour invasion and metastasis and, in particular, proved to be a significant prognostic factor for node-positive cases. In cases of breast cancer, p53 expression was closely related to prognosis for node-negative cancers (Thor et al, 1992; Allred et al, 1993). However, early stages of gastric cancer showed no lymph node metastasis, and the post-operative prognosis was improved in both p53-negative and -positive groups by the surgical treatment (Maehara et al, 1992b, 1993).

Other investigators reported that p53 overexpression is not related to the prognosis of human malignancies, including gastric cancer (Motojima et al, 1994; Schneider et al, 1994; Gabbert et al, 1995). McLaren et al (1992) suggested that p53 was of considerable importance in the initiation of tumours in a wide variety of tissues, but the nature of the particular oncogene involved initially is probably of little significance once a tumour has developed. p53 protein expression could also be induced by a number of other factors, i.e. viral infection, oncogene overexpression and transcriptional activation, or mutations outside the conserved regions of p53 (Wynford-Thomas, 1992; Bell et al, 1993). There are reports of a combined assessment of expressions of p53 and of other factors, for example, cyclin E and vascular endothelial growth factor as useful factors for evaluating tumour behaviour and the poor post-operative prognosis for subjects with various cancers (Kang et al, 1997; Furihata et al, 1998; Sakaguchi et al, 1998; Takahashi et al, 1998). Therefore, these approaches may further the clinical usefulness of examining p53 expression.

The present findings show that p53 expression is closely related to tumour invasion and metastasis and a poorer prognosis in humans with gastric cancer. Because p53 is an integral part of anticancer-related DNA damage and apoptotic pathways (Caelles et al, 1994; Carson and Lois, 1995), the clonal expansion of cells that acquire mutations in the p53 gene reveals resistance to cancer chemotherapy of solid tumours (Lowe et al, 1994). Post-operative chemotherapy proved to be non-effective for p53-negative breast cancer, therefore accumulation of p53 protein can lead to enhanced chemoresistance (Koechli et al, 1994; Elledge et al, 1995). Chin et al (1992) reported that the multidrug resistance gene could be activated during the tumour progression associated with mutations in p53. Almost all of our patients with advanced gastric cancer were prescribed post-operative chemotherapy, therefore the relation between p53 protein expression and the effect of post-operative chemotherapy could not be determined. An appropriate treatment for patients with gastric cancer with p53 expression has yet to be determined.
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REFERENCES

Allred DC, Clark GM, Elledge R, Fuqua SA W, Brown RW, Chamness GC, Osborne CK and McGuire WL (1993) Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. J Natl Cancer Inst 85: 200–206

Banks L, Matlashewski M and Crawford L (1986) Isolation of human-p53-specific monoclonal antibodies and their use in the studies of human p53 expression. Eur J Biochem 159: 529–534

Bell SM, Scott N, Cross D, Sagar P, Lewis FA, Blair GE, Taylor GR, Dixon MF and Quirke P (1993) Prognostic value of p53 overexpression and c-Ki-ras gene mutations in colorectal cancer. Gastroenterology 104: 57–64

Bozzetti F, Bonfanti G, Bufalino R, Menotti V, Persiani S, Andreola S, Doci R and Gennari L (1982) Adequacy of margins of resection in gastric cancer. Ann Surg 196: 685–690

Brambilla E, Gazzeri S, Moro D, De Fromentel CC, Gouyer V, Jacquot M and Brambilla C (1993) Immunohistochemical study of p53 in human lung carcinomas. Am J Pathol 143: 199–210

Caelles C, Helberg A and Karin M (1994) p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature 370: 220–223

Canson DA and Lois A (1995) Cancer progression and p53. Lancet 346: 1009–1011

Chang F, Styrján S and Styrján K (1995) Implications of the p53 tumor suppressor gene in clinical oncology. J Clin Oncol 13: 1009–1022

Chin K-V, Ueda K, Pastan I and Gottesman MH (1992) Modulation of activity of the promoter of the human MDR1 gene by ras and p53. Science 255: 459–462

Cox DR (1972) Regression models and life tables. J R Stat Soc Series B 34: 187–220

Cross SM, Sanchez CA, Morgan CA, Schirme M, Ramel S, izderza RL, Raskind WH and Reid BJA (1995) A p53-dependent mouse spindle checkpoint. Science 267: 1353–1356

Dixon WJ (1988) BMDP Statistical Software. University of California Press: Berkeley, CA

Elledge RM, Gray R, Mansour E, Yu Y, Clark GM, Ravdin P, Osborne CK, Gilchrist D, Dixon WJ (1988) Association of p53 and vascular endothelial growth factor expression in colon cancer specimens. Lancet 344: 1647–1648

Kawasaki S (1975) A clinicopathological study on upward intramural extension of cancer of the stomach (in Japanese with English abstract). Fukuoka Acta Med 66: 1–23

Kim J-H, Takahashi T, Chiba I, Park J-G, Birrer MJ, Roh JK, Lee HD, Kim J-P, Minna JD and Gazdar AF (1991) Occurrence of p53 gene abnormalities in gastric cancer tumors and cell lines. J Natl Cancer Inst 83: 938–943

Kochel DE, Scher GA, Seiffert B, Hornung R, Hulle H, Eppenberger U and Muller H (1994) Mutant p53 protein associated with chemosensitivity in breast cancer specimens. Lancet 344: 1647–1648

Korenaga D, Okamura T, Baba H, Saito A and Sugimachi K (1989) Results of resection of gastric cancer extending to adjacent organs. Br J Surg 75: 125–128

Levine AJ, Momand J and Finlay CA (1991) The p53 tumour suppressor gene. Nature 351: 435–436

Levine AJ, Perry ME, Chang A, Silver A, Dittmer D, Wu M and Welsh D (1994) The role of the p53 protein in senescence and tumor suppression. Science 264: 809–817

Maehara Y, Okumura T, Moriguchi S, Orita H, Kusumoto H, Korenaga D and Sugimachi K (1992a) Prophylactic lymph node dissection in patients with advanced gastric cancer promotes increased survival time. Cancer 70: 392–395

Maehara Y, Orita H, Okumura T, Moriguchi S, Tsujitani S, Korenaga D and Sugimachi K (1992b) Predictors of lymph node metastasis in early gastric cancer. Br J Surg 79: 245–247

Maehara Y, Okumura T, Oshiro T, Baba H, Anai H, Akazawa K and Sugimachi K (1993) Early carcioma of the stomach. Surg Gynecol Obstet 177: 593–597

Maehara Y, Okumura T, Kakeji Y, Endo K, Yamamoto M and Sugimachi K (1995) A tumour-associated cell-surface glycoprotein accompanying p53 overexpression and higher growth potential for gastric cancer. Br J Cancer 71: 999–1002

Martin HM, Filipe MJ, Morris RW, Lane DP and Silvestre F (1992) p53 expression and prognosis in gastric carcinoma. Int J Cancer 50: 859–862

McLaren R, Kuzu I, Dummil M, Harris A, Lane D and Gutter KC (1992) The relationship of p53 immunostaining to survival in carcinoma of the lung. Br J Cancer 66: 735–738

McLaren SP, Eich S, Zaitas TK, Spedding T, Balduin SE and Pichlmair H (1997) p53 expression in gastric cancer. Clinicoopathological correlation and prognostic significance. Dig Dis Sci 42: 2463–2467

Mototjima K, Furui J, Kohara N, Ito T and Kanematsu T (1994) Expression of p53 protein in gastric carcinomas is not independently prognostic. Surgery 116: 890–895

Narayana A, Vaughan AT, Gunaratne S, Kathuria S, Walter SA and Reddy SP (1989) Is p53 an independent prognostic factor in patients with laryngeal carcinoma? Cancer 82: 286–291

Oiwa H, Maehara Y, Ohno S, Sakaguchi Y, Ichiyoshi Y and Sugimachi K (1995) Growth pattern and p53 expression in patients with early gastric cancer. Cancer 75: 1454–1459

Pilkif J, Bánkfalvi A, Tory K, Filész L, Bryne M, Öfner D, Kusch F, Joos U and Schmid KW (1998) Molecular assessment of p53 abnormalities at the invasive front of squamous cell carcinomas. Head Neck 20: 8–15

Porter PL, Gown AM, Kram SG and Colterrnea MD (1992) Widespread p53 overexpression in human malignant tumors. Am J Pathol 140: 145–153

Quinlan DC, Davidson AG, Summers CL, Warden HE and Doshi HM (1992) Accumulation of p53 protein correlates with a poor prognosis in human lung cancer. Cancer Res 52: 4828–4831

Sakaguchi T, Watanabe A, Sawada H, Yamada Y, Yamashita J, Matsuda M, Nakajima M, Miwa T, Hiroa T and Nakano H (1998) Prognostic value of cyclin E and p53 expression in gastric carcinoma. Cancer 82: 1238–1243

SARBIA M, Porschen R, Borchard F, Horstmann O, Willers R and Gehrke HE (1994) p53 protein expression and prognosis in squamous cell carcinoma of the esophagus. Cancer 74: 2218–2223

Schneider BG, Hilsenbeck SG, Demel CH, Sokel V, Shelton CH, Rodriguez-Martinez HA, Gutierrez-Diaz CME, Pultiter DR and Allred DC (1994) p53 mutations in gastric and colorectal cancers in Texas Hispanics versus Anglos. Virchows Arch 424: 187–193

Kakeji Y, Korenaga D, Tsujitani S, Baba H, Anai H, Maehara Y and Sugimachi K (1993) Gastric cancer with p53 overexpression has high potential for metastasising to lymph nodes. Br J Cancer 67: 589–593

Kang S-M, Maeda K, Onoda N, Chung Y-S, Nakata B, Nishiguchi Y and Sowa M (1997) Combined analysis of p53 and vascular endothelial growth factor expression in colorectal carcinoma for determination of tumor vascularity and mortality metastasis. Int J Cancer 74: 502–507

Japanese Research Society for Gastric Cancer (ed) (1993) The General Rules for Gastric Cancer Study (in Japanese), 12th edn. Kanehara and Co.: Tokyo, Japan

Joypaul BV, Hopwood D, Newman EL, Qureshi S, Grant A, Ogston SA, Lane DP and Cuschiari A (1994) The prognostic significance of the accumulation of p53 tumour-suppressor gene protein in gastric adenocarcinoma. Br J Cancer 69: 943–946

Japanese Research Society for Gastric Cancer (ed) (1993) The General Rules for Gastric Cancer Study (in Japanese), 12th edn. Kanehara and Co.: Tokyo, Japan

Joypaul BV, Hopwood D, Newman EL, Qureshi S, Grant A, Ogston SA, Lane DP and Cuschiari A (1994) The prognostic significance of the accumulation of p53 tumour-suppressor gene protein in gastric adenocarcinoma. Br J Cancer 69: 943–946
Shimaya K, Shiosaki H, Inose M, Tahara H, Monden T, Shimano T and Mori T (1993) Significance of p53 expression as prognostic factor in oesophageal squamous cell carcinoma. *Virchows Arch A Pathol Anat* 422: 271–276

Sobin LH and Wittekind C (1997) *TNM Classification of Malignant Tumour*, 5th edn. John Wiley & Sons: New York, NY

Soussi T, Legros Y, Lubin R, Ory K and Schlichtholz B (1994) Multifactorial analysis of p53 alteration in human cancer: a review. *Int J Cancer* 57: 1–9

Starzynska T, Bromley M, Ghosh A and Stern PL (1992) Prognostic significance of p53 overexpression in gastric and colorectal carcinoma. *Br J Cancer* 66: 558–562

Sugimachi K, Maehara Y, Ogawa M, Kakegawa T and Tomita, M (1997) Dose intensity of uracil and tegafur in postoperative chemotherapy for patients with poorly differentiated gastric cancer. *Cancer Chemother Pharmacol* 40: 233–238

Suto T, Sugai T, Nakamura S, Funato O, Nitta H, Sasaki R, Kanno S and Saito K (1998) Assessment of the expression of p53, MIB-1 (Ki-67 antigen), and argyrophilic nucleolar organizer regions in carcinoma of the extrahepatic bile duct. *Cancer* 82: 86–95

Takahashi Y, Bucana CD, Cleary KR and Ellis LM (1998) p53, vessel count, and vascular endothelial growth factor expression in human colon cancer. *Int J Cancer* 79: 34–38

Thor AD, Moore II DH, Edgerton SM, Kawasaki ES, Reihsaus E, Lynch HT, Marcus JN, Schwartz L, Chen L–C, Mayall BH and Smith HS (1992) Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis in breast cancers. *J Natl Cancer Inst* 84: 845–855

Uchino S, Noguchi M, Ochiai A, Saito T, Kobayashi M and Hirohashi S (1993) p53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. *Int J Cancer* 54: 759–764

Vogelstein B and Kinzler KW (1992) p53 function and dysfunction. *Cell* 70: 523–526

Wynford-Thomas D (1992) p53 in tumour pathology: can we trust immunocytochemistry? *J Pathol* 166: 329–330

Yamaguchi A, Nakagawara G, Kurosaka Y, Nishimura G, Yonemura Y and Miyazaki I (1993) p53 immunoreaction in endoscopic biopsy specimens of colorectal cancer, and its prognostic significance. *Br J Cancer* 68: 399–402

Yokozaki H, Kuniyasu H, Kitadai Y, Nishimura K, Todo H, Ayhan A, Yasui W, Ito H and Tahara E (1992) p53 point mutations in primary gastric carcinomas. *J Cancer Res Clin Oncol* 119: 67–70