Preoperative Treatment with Tegafur Suppositories Enhances Apoptosis and Reduces the Intratumoral Microvessel Density of Human Colorectal Carcinoma

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Supported in part by Grants-in-Aid for Cancer Research from the Minstrry of Education, Science, Sports, and Culture of Japan.

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Received January 11, 1999; revisions received June 1, 1999, and September 23, 1999; accepted September 23, 1999.

BACKGROUND. This study examined the effect of tegafur, a depot of 5-fluorouracil, in human colorectal carcinomas in terms of apoptosis, cell proliferation, and expression of p53 gene and angiogenesis-related molecules.

METHODS. A total of 32 patients with colorectal carcinoma were divided into 2 groups; 20 patients received tegafur suppositories (TS) at 1 g/day for 14 days before surgery, and 12 patients did not receive any chemotherapy. Surgically removed specimens were examined immunohistochemically for Ki-67, CD34, p53, p21, Bax, vascular endothelial growth factor (VEGF), and thymidine phosphorylase (dThdPase). Apoptotic tumor cells were visualized by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick-end labeling (TUNEL) procedure.

RESULTS. The mean percentage of apoptotic index (AI) was 6.9 ± 1.2 in the 20 TS-treated tumors and 4.4 ± 1.0 in the 12 nontreated tumors ($P < 0.001$). In contrast, the mean percentage of Ki-67 labeling index (KI) became significantly lower in the former group ($P < 0.05$). The frequency of p21 expression was significantly higher in the TS-treated group than in the nontreated group ($P < 0.05$), whereas no difference was detected in p53 and Bax expression between the two groups. The mean intratumoral microvessel density was 47.8 ± 19.8 in the TS-treated tumors and 66.8 ± 16.5 in the nontreated tumors ($P < 0.01$). The frequency of dThdPase expression, but not of VEGF expression, became significantly lower with the TS treatment. p53 expression did not correlate with AI, KI, IMV density, or the expression of VEGF, p21, or Bax, except for dThdPase, which was significantly higher in the 18 p53 positive tumors ($P < 0.05$).

CONCLUSIONS. Preoperative TS treatment enhances apoptosis and suppresses angiogenesis of colorectal carcinomas in a p53-independent manner. ***Cancer* 2000; 88:1007–15.** © *2000 American Cancer Society.*

KEYWORDS: tegafur suppository, colorectal carcinoma, apoptosis, angiogenesis, p53 gene.

5-Fluorouracil (5-FU) is most commonly used in the treatment of gastrointestinal, breast, and lung carcinomas. The mechanism of action of 5-FU is now considered to involve its metabolic conversion to 5-fluorouridine 5′-triphosphate (5-FUTP) with subsequent incorporation into RNA, and/or the formation of 5-fluoro-2′-deoxyuridine 5′-monophosphate (5-FdUMP), a well-known inhibitor of thymidylate synthetase.[1]

Nabeya et al. and Pickard et al. reported that 5-FU induced expression of the wild-type p53 gene, followed by the induction of transcription of a variety of genes, including transcriptional factors such as Bax and p21; this resulted in arrest of cell growth and induc-

tion of apoptosis.[2,3] We demonstrated that 5-FU induced apoptosis in human gastric carcinoma cell lines MKN-45 and MKN-74, which carry the wild-type p53 gene, but not in MKN-28 (which carries a mutated p53 gene) or in KATO-III (which lacks the gene).[4] MKN-45 and MKN-74 showed a gradual increase of the Bax/Bcl-2 expression ratio during the process of apoptosis. Thus, the p53 gene may play a crucial role in the induction of apoptosis by 5-FU. More recently, expression of the p53 gene has been demonstrated to be related to intratumoral angiogenesis. A mutated p53 gene has been shown to be a potent stimulant of vascular endothelial growth factor (VEGF).[5] These observations led us to consider that a mutated p53 gene provides an advantage for tumor proliferation not only by allowing escape from apoptosis, but also by leading to formation of a vascular-rich microenvironment.

5-FU suppositories were first used to treat patients with rectal cancer by Takahashi et al., who demonstrated a high concentration of 5-FU in cancer tissue[6] and found that combined preoperative radiochemotherapy using 5-FU suppositories produced shrinkage of tumor size in 18 of 23 patients and tumor disappearance in 2 of 23 patients.[6,7] The effects were strongest when preoperative treatments combining radiation, intraluminal hyperthermia, and 5-FU suppositories were applied.[8] They found an apparent reduction in tumor size macroscopically and a marked decrease in the number of cancer cells, with total absence of cancer cells in 3 of 30 cases, microscopically. However, they did not discuss the precise mechanism of 5-FU in the abatement of rectal carcinomas.

Recently, Kaibara et al. reported that preoperative continuous intravenous administration of 5-FU at 500 mg/body/day for 7 days significantly increased the number of apoptotic cancer cells in patients with gastric and colorectal carcinomas, the effect being more obvious in the latter.[9,10] No examination, however, was made of intratumoral angiogenesis.

We therefore examined the effect of preoperative treatment with TS (which acts as a depot form of 5-FU) in human colorectal carcinomas in terms of apoptosis, cell proliferation, expression of angiogenesis-related molecules, and intratumoral microvessel density (IMVD), and the effect on p53 gene expression. Our results may provide an indication of the potential for clinical usefulness of preoperative treatment of patients with colorectal carcinoma with TS.

MATERIALS AND METHODS

Patients and Specimens

Thirty-two patients were preoperatively diagnosed with advanced colorectal carcinoma at Hiroshima Prefectural Hospital in 1997 and 1998. Among them, 20 patients received TS at 1 g/day for 14 days before operation, but no other treatment, and the others did not receive chemotherapy. These protocol were based on an experimental model and human analysis to clarify that preoperative 5-FU or tegafur administration is necessary for at least 7 days.[11,12] Informed consent was obtained from the all patients before the TS treatment.

The two groups were closely matched and there were no differences in their clinicopathologic backgrounds (Table 1) with regard to age, gender, localization, histologic type of disease, or Dukes classification. For example, histology of the well-differentiated type occurred in 17 of the tegafur-treated group and in 10 of the nontreated group. A survey of family history disclosed no cancer patients among the all patients' family members, except for 2 (Case No. 4 and 29), whose fathers developed rectal carcinomas when they were older than 60 years. Thus, none of the cases fulfilled the Amsterdam criteria for hereditary nonpolyposis colorectal carcinoma.[13]

Surgically removed specimens were fixed in 15% buffered formalin for 1 day and embedded in paraffin. One or two representative blocks, including the main lesion, were selected, and serial sections thereof were examined by light microscopy, immunohistochemistry, and the terminal deoxynucleotidyl transferase–mediated dUTP-digoxigenin nick-end labeling (TUNEL) procedure.

Immunohistochemistry

Dewaxed paraffin sections were immunostained by the streptavidin-biotin peroxidase complex (SAB) method. The following primary antibodies were used: antibodies raised against Ki-67 (MIB-1, diluted 1:100; Immunotech, Marseille, France); CD34 (CD34 [NU-4A1]; Nichirei, Japan); VEGF (VEGF [A-20], diluted 1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA); p53 (BP53-12, diluted 1:50; Novocastra Laboratories Ltd., Newcastle, UK); p21 (p21 [187], diluted 1:1000; Santa Cruz Biotechnology, Inc.); Bax (Bax [p-19], diluted 1:200; Santa Cruz Biotechnology, Inc.); dThdPase (dThdPase [654-1], diluted 1:1000; Nippon Roche Research center, Kanagawa, Japan). Trypsin pretreatment was performed for Ki-67 staining. All the sections were heated in 10 mM pH 6.0 citrate buffer in a microwave oven for 15 minutes at 94 °C. Immunoreactions were visualized with diaminobenzidine (DAB) and the sections were counterstained with 3% methyl green.

To examine the specificity of immunostaining, the primary antibody was replaced by mouse normal immunoglobulin G at a 1:100 dilution and Tris-buffered saline. Control slides were invariably negative for im-

TABLE 1
Clinical Features, AI, KI, p53, p21, Bax, IMVD, VEGF, and dThdPase Expression of the 32 Colorectal Carcinoma Tissue Samples Analyzed

Case no.	Age (yrs)	Gender	Localization	Histologic type[a]	Clinical stage (Dukes)	AI (%)	KI (%)	p53	p21	Bax	IMVD	VEGF	dThdPase
TS-treated patients													
1	70	F	Colon	Well	B	6.2	77.1	+	+	+	51	−	+
2	71	F	Colon	Well	C	7.0	86.9	+	−	−	53	+	−
3	53	M	Rectum	Well	A	8.5	57.8	+	+	−	58	−	−
4	83	F	Colon	Well	B	6.5	75.9	+	+	−	24	+	−
5	78	M	Colon	Well	B	6.8	59.5	+	−	−	66	+	+
6	69	F	Colon	Well	B	7.2	49.6	+	−	−	40	−	−
7	71	F	Colon	Mod	C	6.8	64.0	+	+	+	48	+	−
8	67	M	Rectum	Well	C	8.0	67.2	+	−	+	38	+	+
9	51	F	Colon	Mod	C	8.0	85.0	+	−	+	68	+	−
10	62	M	Colon	Poor	B	5.5	65.0	+	−	−	20	−	+
11	68	F	Rectum	Well	C	6.4	68.0	+	+	+	38	−	−
12	60	M	Colon	Well	B	8.1	78.5	−	+	+	45	+	−
13	50	M	Rectum	Well	B	6.7	94.6	−	+	+	41	+	−
14	69	M	Rectum	Well	A	4.8	25.0	−	−	+	99	+	−
15	71	F	Colon	Well	A	5.5	39.4	−	+	+	64	+	+
16	76	M	Rectum	Well	A	6.5	65.0	−	+	+	30	+	−
17	75	M	Rectum	Well	A	4.9	80.0	−	−	−	32	−	−
18	85	M	Rectum	Well	B	9.0	61.8	−	−	−	75	−	−
19	58	M	Rectum	Well	B	9.1	72.0	−	+	+	21	+	−
20	45	M	Colon	Well	C	7.1	75.0	−	+	+	45	+	−
Untreated patients													
21	73	M	Rectum	Well	A	1.6	62.8	+	−	−	83	+	−
22	60	M	Colon	Well	A	4.8	80.2	+	−	+	71	+	+
23	88	F	Colon	Mod	B	4.0	82.4	+	+	+	46	−	+
24	79	F	Rectum	Well	B	5.0	83.2	+	−	+	79	+	+
25	80	M	Colon	Well	A	4.8	82.4	+	−	−	95	−	+
26	50	M	Rectum	Well	C	5.2	75.0	+	−	+	64	+	+
27	71	M	Rectum	Mod	B	4.8	78.0	+	−	+	51	+	−
28	46	F	Colon	Well	B	3.3	60.4	−	+	−	64	−	−
29	47	M	Rectum	Well	A	4.9	79.2	−	−	−	40	+	−
30	81	F	Colon	Well	B	4.2	87.6	−	−	−	55	+	−
31	42	F	Colon	Well	C	5.2	81.0	−	−	−	74	+	+
32	48	F	Rectum	Well	B	4.7	82.6	−	−	+	80	+	+

AI: apoptotic index; KI: Ki-67 labeling index; IMVD: intratumoral microvessel density; VEGF: vascular endothelial growth factor; dThdPase: thymidine phosphorylase.
[a] Well: well-differentiated adenocarcinoma; Mod: moderately differentiated adenocarcinoma; Poor: poorly differentiated adenocarcinoma.

munostaining. We immunostained appropriate control slides of colonic carcinoma, which were previously known to show the positive immunoreactivity for Ki-67, CD34, p53, p21, Bax, VEGF, and dThdPase, and at the same time to obtain constant findings.

TUNEL Method

To detect DNA breakage in situ, TUNEL was performed according to the method of Gavrieli et al.,[14] using Apop Tag Plus in situ apoptosis detection kits (Oncor, Inc., Gaithersburg, MD). Briefly, after deparaffinization, incubation with 20 μg/mL of proteinase K (Boehringer Mannheim/Yamanouchi, Tokyo, Japan) for 40 minutes at 37 °C and blocking of endogenous peroxidase with 2% hydrogen peroxidase (H_2O_2) in methanol for 30 minutes at room temperature was performed. After prehybridization treatment, the sections were exposed to terminal deoxynucleotidyl transferase with digoxigenin-11-dUTP and dATP and incubated in a moist chamber for 90 minutes at 37 °C. Anti-digoxigenin-antibody-peroxidase was applied for 30 minutes at room temperature for detecting digoxigenin-11-dUTP labeling, followed by color development with an H_2O_2 solution containing 3,3′-diaminobenzidine. Methyl green was used for counterstaining.

Evaluation of TUNEL Positive and Ki-67 Positive Cells

The apoptotic index (AI) of each colorectal carcinoma was obtained as the ratio of the number of TUNEL positive cancer cells per 1000 cancer cells in the most frequently labeled area. Ki-67 labeling indices (KIs)

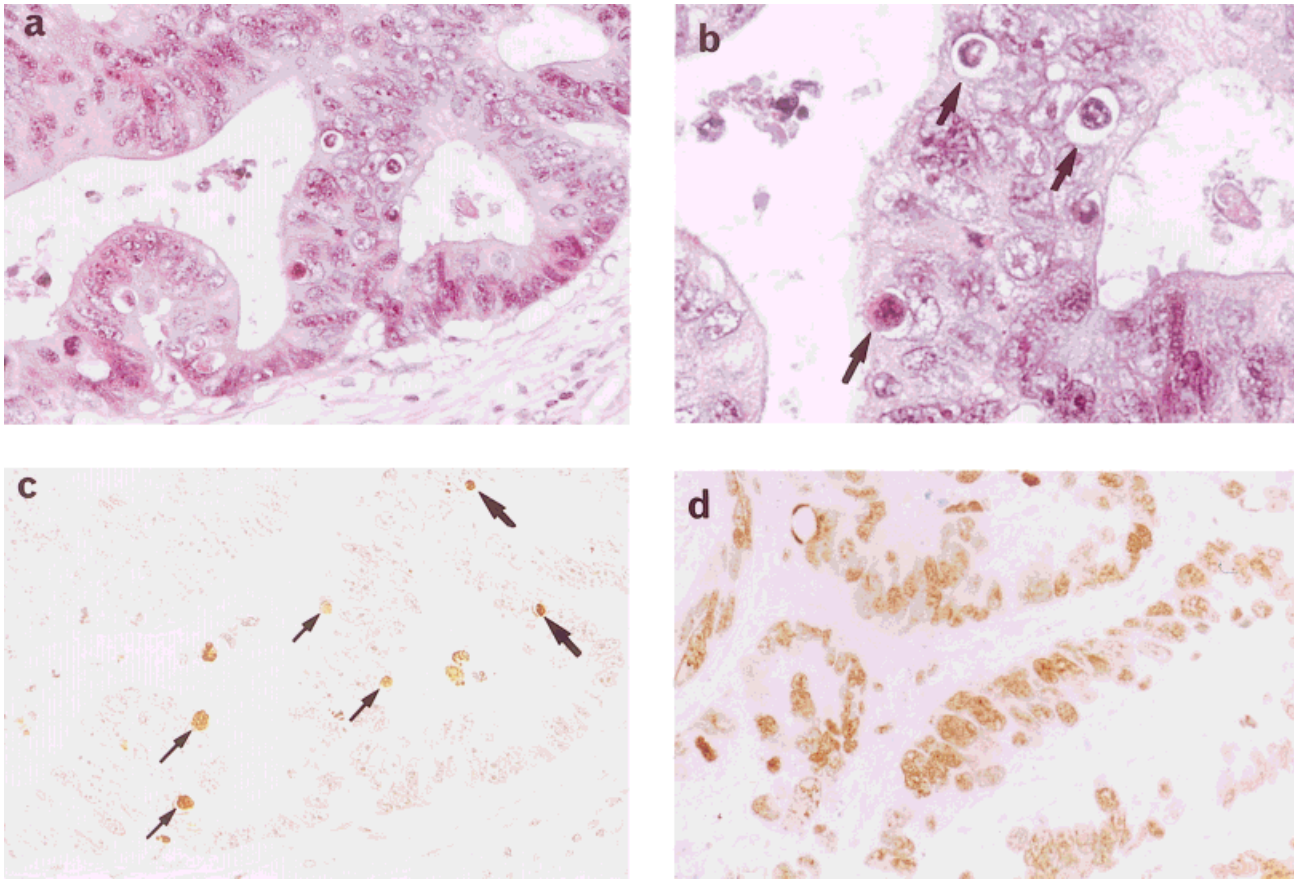

FIGURE 1. (a) Colorectal adenocarcinoma from a patient treated with TS is shown. A few apoptotic cells are detected. Apoptotic bodies showing fragmented nuclei are seen in the lumen of cancer glands (H&E, original magnification ×240). (b) Higher magnification of Figure 1a. Apoptotic cells are indicated by arrows (H&E, original magnification ×480). (c) TUNEL signals are observed in the nuclei of apoptotic cancer cells (small arrows) and in normal-looking, nonpyknotic cancer cells (large arrows) (TUNEL staining, original magnification ×240; semiserial section as in Fig. 1a). (d) Numerous cancer cells show nuclear immunoreactivity for Ki-67 (immunohistochemical staining, original magnification ×240).

were also calculated in the same manner as the TUNEL index.

Assessment of VEGF, p53, p21, Bax, and dThdPase Immunostaining

We judged samples to be positive for VEGF, p53, p21, Bax, or dThdPase when at least 10% of cancer cells were specifically stained.

Determination of IMVD

For the determination of IMVD using CD34, at least 10 of the most vascularized areas within a section were selected and counted under a light microscope with a 200-fold magnification, as described by Weidner et al.[15] The average numbers were recorded as the IMVD for each case. The IMVD was counted by two authors (T.M. and H.I.) independently without knowledge of the clinical information, including the treatment; then the results were adjusted.

Statistical Analysis

The Student *t* test was used for statistical analysis. A P value <0.05 was considered significant.

RESULTS

TUNEL signal positive apoptotic cancer cells were detected in both TS-treated and nontreated tumors. Hematoxylin and eosin (H&E) staining of serial sections revealed various types of TUNEL positive cells, including 1) cells with nuclear condensation and slightly eosinophilic cytoplasm, 2) cells with fragmented nucleus, and 3) normal-looking cells (Fig. 1a–c). Fragmented nuclei corresponding to apoptotic bodies were frequently detected in the lumen of the cancer glands. A very few TUNEL positive apoptotic cells were found in the deeper portion of the adjacent normal glands in the TS-treated specimens, but none were found in the nontreated specimens.

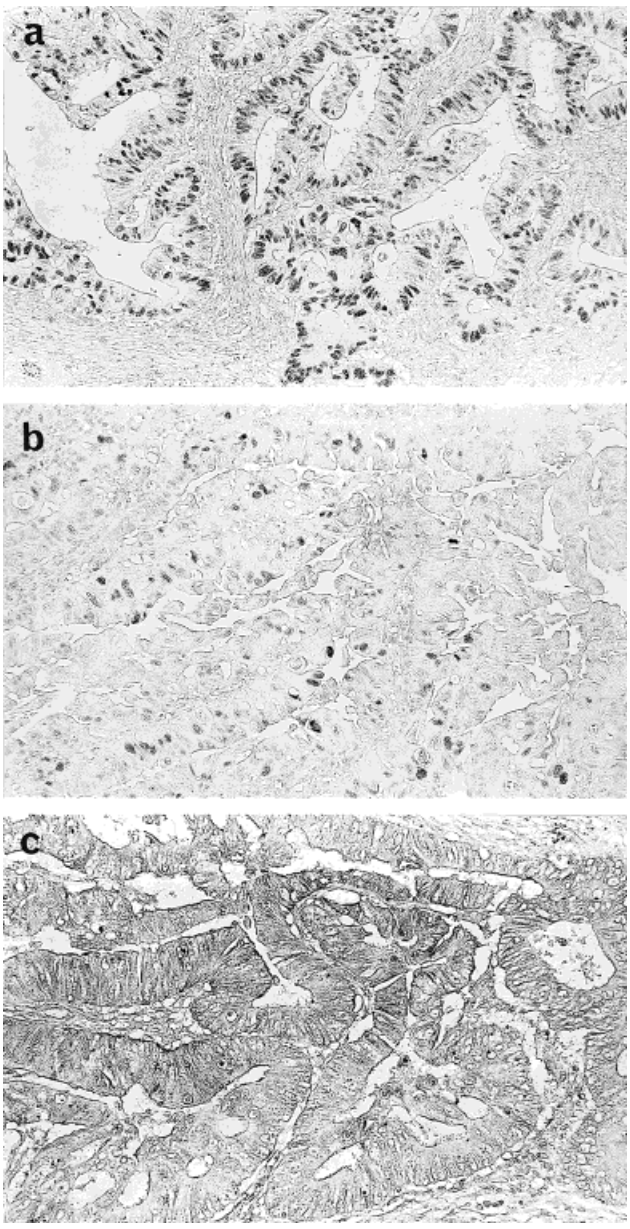

FIGURE 2. Immunohistochemical staining for p53 (a), p21 (b), and Bax (c) is shown in cancer tissues of TS-treated patients (immunohistochemical staining, original magnification ×120).

Ki-67, p53, and p21 immunoreactivities were localized in the nuclei of cancer cells without more specific distribution (Fig. 1d, Figure 2a and b), whereas Bax was found in the cytoplasm of cancer cells (Fig. 2c). CD34 immunoreactivity was found in the cytoplasm of the endothelium in both the normal and tumoral portion and clearly revealed the presence of intratumoral microvessels (Fig. 3a and b). VEGF immunoreactivity was found mainly in the cytoplasm of tumor cells, especially in the front of invasion, but not in the normal colorectal mucosal cells (Fig. 3c). Weak immunostaining was noted in a few endothelial cells. dThdPase was demonstrated in both cancer cells and stromal cells, including macrophages and fibroblasts (Fig. 3d).

Table 2 summarizes the measurement of the mean AI, KI, and IMVD and expression of p53, p21, Bax, VEGF, and dThdPase. The mean AI was 6.9 ± 1.2 in the 20 TS-treated tumors and 4.4 ± 1.0 in the 12 nontreated tumors, and the difference between these values was significant ($P < 0.001$). In addition, the mean KI was 67.4 ± 16.3 in the TS-treated tumors and 77.9 ± 8.2 in the nontreated tumors, and these values were also significantly different ($P < 0.05$). These results suggested that preoperative TS treatment enhanced apoptosis and inhibited cell proliferation of the colorectal carcinomas. The frequency of p21 expression was significantly higher in the TS-treated tumors than in the nontreated tumors ($P < 0.05$), whereas no difference was detected between p53 and Bax expression of the two groups.

The mean IMVD was 47.8 ± 19.8 in the TS-treated tumors and 66.8 ± 16.5 in the nontreated tumors, the value being significantly lower in the former than in the latter group ($P < 0.01$). Expression of dThdPase was detected in 5 (25%) of the TS-treated tumors and in 6 (58%) of the nontreated tumors. The frequency was significantly lower in the former ($P < 0.05$). VEGF expression was found in 13 (65%) of the TS-treated tumors and in 9 (75%) of the nontreated tumors, and these frequencies were not significantly different.

Next, we divided the tumors into two categories, p53 positive and P53 negative, in order to analyze the role of the p53 gene in the induction of apoptosis (Table 3). There was no significant difference between the mean values of AI (6.0 ± 1.7 vs. 6.0 ± 1.8), KI (72.2 ± 10.8 vs. 70.2 ± 18.9), or IMVD (55.2 ± 19.9 vs. 54.6 ± 22.2) or the frequencies of p21, Bax, or VEGF expression in the two groups; however, the dThdPase expression was significantly higher in the p53 positive tumors ($P < 0.05$).

The mean AI was significantly higher in the group receiving TS treatment ($P < 0.001$). The mean KI and IMVD were significantly decreased by TS treatment in the p53 positive tumors ($P < 0.05$). Although the mean KI and IMVD tended to be lower after the TS treatment in the p53 negative tumors, there were no significant differences. This might have been partly due to the small number of p53 negative tumors in this study. Expression of Bax was significantly more frequent in the TS-treated tumors than in the nontreated tumors among the p53 negative tumors, but not among the p53 positive tumors (Table 3).

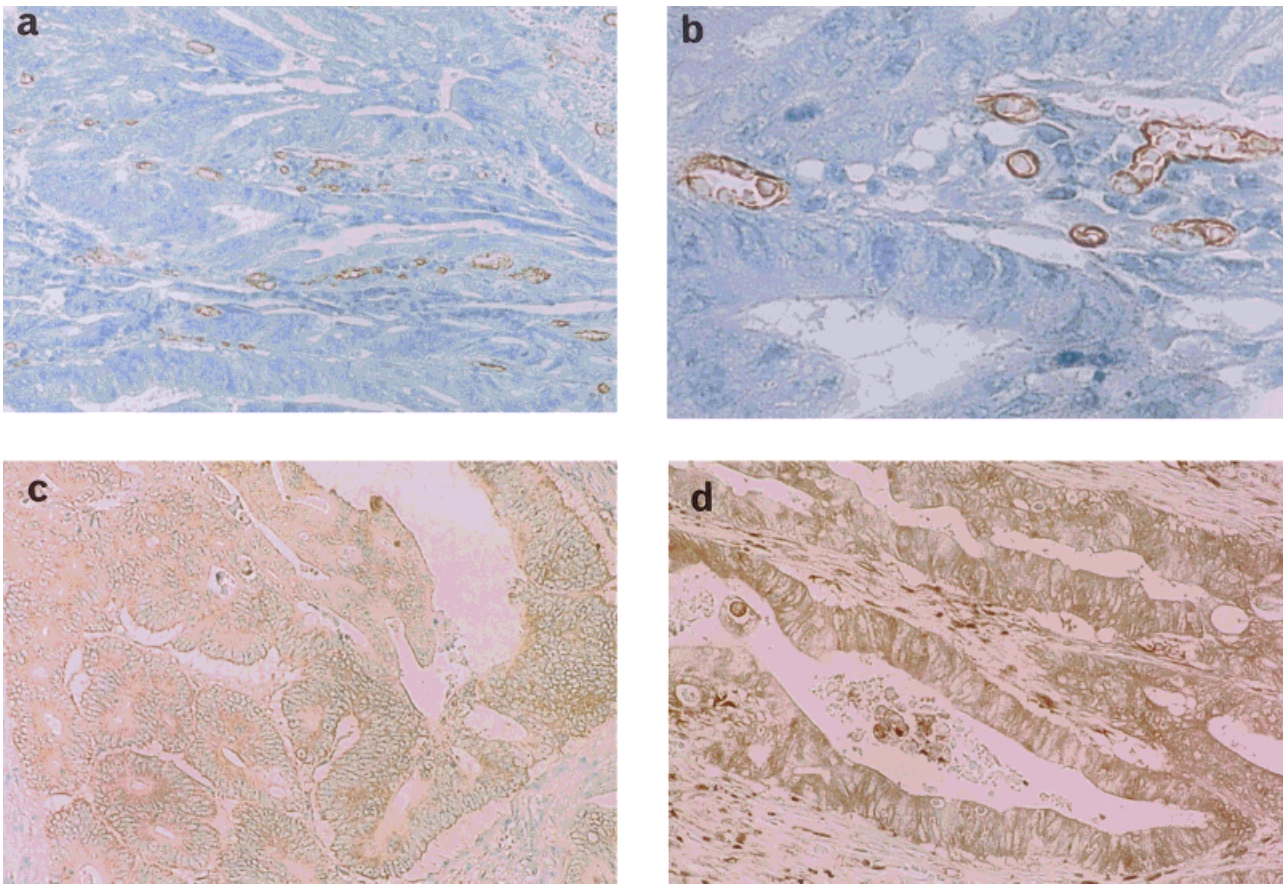

FIGURE 3. (a) Immunohistochemical staining for CD34 is shown in cancer tissues of TS-treated patients. Intratumoral microvessels are clearly demonstrated (immunohistochemical staining, original magnification ×120). (b) Higher magnification of Figure 3a (Immunohistochemical staining, original magnification ×480). (c) Immunohistochemical staining for vascular endothelial growth factor is shown in cancer tissues of TS-treated patients (immunohistochemical staining, original magnification ×120). (d) Immunohistochemical staining for dThdPase is shown in cancer tissues of TS-treated patients. Immunoreactivity are shown both in the most of cancer cells and in a good number of stromal cells (immunohistochemical staining, original magnification ×120).

TABLE 2
Apoptosis, IMVD, and Expression of Ki-67, p53, p21, Bax, VEGF, and dThdPase in 32 Colorectal Carcinomas

	Mean AI	Mean KI	p53	p21	Bax	IMVD	VEGF	dThdPase
Tegafur-treated group (n = 20)	6.9 ± 1.2[a]	67.4 ± 16.3	11 (55%)	11 (55%)	12 (60%)	47.8 ± 19.8	13 (65%)	5 (25%)
Control group (n = 12)	4.4 ± 1.0	77.9 ± 8.2	7 (58%)	2 (17%)	6 (50%)	66.8 ± 16.5	9 (75%)	6 (58%)
P value	<0.001	0.011	0.430	0.011	0.300	0.003	0.281	0.038

AI: apoptotic index; KI: Ki-67 labeling index; IMVD: intratumoral microvessel density; VEGF: vascular endothelial growth factor; dThdPase: thymidine phosphorylase.
[a] Values are expressed as mean ± standard error.

DISCUSSION

In the current study, we clearly demonstrated that preoperative administration of TS for 14 days significantly enhanced the apoptosis and abated the IMVD of colorectal carcinomas. In other words, TS might suppress the tumor proliferation not only by the direct effect of increasing the apoptosis of cancer cells, but also by the indirect effect of reducing the blood supply to the cancer cells. In fact, mild ischemia is known to enhance apoptosis in a variety of cells.[16]

Apoptosis was initially defined by the morphologic criteria of Kerr.[16] Clear histology of apoptotic cancer cells was seen in our previous studies of apoptotic cell death in human gastric and colonic carci-

TABLE 3
Relation between Nuclear p53 Expression and AI, Ki-67, p21, Bax, IMVD, VEGF, dThdPase in 32 Colorectal Carcinomas

	AI	KI	p21	Bax	IMVD	VEGF	dThdPase
p53 positive group							
Tegafur-treated (n = 11)	7.0 ± 0.9[a]	68.7 ± 11.5	5 (45%)	5 (45%)	45.8 ± 15.6	6 (55%)	4 (36%)
Control group (n = 7)	4.3 ± 1.3	77.7 ± 7.2	1 (14%)	5 (71%)	69.9 ± 17.6	5 (71%)	5 (71%)
P value	<0.001	0.029	0.081	0.150	0.006	0.240	0.083
	6.0 ± 1.7	72.2 ± 10.8	6 (33%)	10 (56%)	55.2 ± 19.9	11 (61%)	9 (50%)[b]
p53 Negative Group							
Tegafur-treated (n = 9)	6.9 ± 1.6	65.7 ± 21.5	6 (67%)	7 (78%)	50.2 ± 24.8	7 (78%)	1 (11%)
Control group (n = 5)	4.5 ± 0.7	78.2 ± 10.4	1 (20%)	1 (20%)	62.6 ± 15.8	4 (80%)	2 (40%)
P value	0.001	0.085	0.053	0.024	0.139	0.460	0.160
	6.0 ± 1.8	70.2 ± 18.9	7 (50%)	8 (57%)	54.6 ± 22.2	11 (79%)	3 (21%)[b]

AI: apoptotic index; KI: Ki-67 labeling index; IMVD: intratumoral microvessel density; VEGF: vascular endothelial growth factor; dThdPase: thymidine phosphorylase.

[a] Values are expressed as mean ± standard error.

[b] $P < 0.05$.

nomas.[17–21] These apoptotic cells showed characteristic features: cell shrinkage, loss of cell-cell contact, and aggregation of the chromatin into dense, often cresent-shaped masses under the nuclear membrane. Apoptotic bodies have occasionally been detected in the tumor gland lumina. Apoptotic cancer cells in tissues, however, are inconspicuous and easily overlooked. Moreover, TUNEL signals were also positive in a few normal-looking cancer cells that might have been in the initial stage of apoptosis.[22] Although some researchers have observed nonspecific labeling using TUNEL methods, apoptotic cells can be assessed by serial TUNEL and H&E stainings in a semiquantitative manner.

As alluded to in the introductory section, the p53 suppressor gene plays a crucial role in the induction of apoptosis. The product of the wild-type p53 gene has been shown to be required for the induction of the apoptotic pathway triggered by oncogenous activation and cytotoxic genes.[23,24] The p53 gene product may sensitize damaged cells to apoptosis, acting to prevent the propagation of transforming mutations. The growth-inhibitory protein p21 is a potent inhibitor of various cyclin-dependent kinases, and the expression of the p21 gene is regulated at the transcriptional level in both a p53 dependent and independent manner.[25] The p21 gene is induced by DNA-damaging agents that trigger G1 arrest in cells carrying the wild-type p53 gene and no mutant p53 gene.[26] Similarly, Bax, whose gene expression is regulated by wild-type p53,[27] is a member of the Bcl-2 family and may act by inhibiting the function of Bcl-2 by forming Bax/Bcl-2 complexes or by competing with other Bcl-2 targets.[28]

Our results seem to be in good agreement with the above-described findings, which have been obtained mainly in primary cell cultures and transgenic mice models. The percentage of p21 positive cells was significantly higher in the group receiving TS treatment. It might be roughly considered that the p53 positive tumors carry mutated p53 genes, whereas the p53 negative tumors carry wild-type p53 genes. Fritsche et al. demonstrated that anticancer agents, such as cisplatin, mitomycin C, etoposide, and 5-FU, as well as energy-rich radiation, act on cellular DNA.[29] These agents induce nuclear accumulation of p53 protein, which is followed by induction of apoptosis in immortalized mouse fibroblasts. Recently, we have demonstrated that the mutated p53 gene attenuates naturally occurring apoptotic cell death in human gastric carcinoma cells.[20] Based on these findings, we had initially expected higher AI in the p53 positive tumors than in the negative tumors. This study, however, could not confirm the significance of the p53 gene status in the induction of apoptosis in colorectal carcinomas. Neither the mean AI nor KI differed between the p53 positive and negative tumors. This might have been partly due to the small number of cases examined, or might imply the predominance of a p53 independent pathway in the induction of apoptosis of human colorectal carcinomas.

Angiogenesis is essential to the growth and progression of various human tumors.[30] The process of intratumoral angiogenesis is regulated by the local balance of various growth factors or cystokines released by both tumor cells and normal cells. In this study, we examined the expression of VEGF and dThdPase, both of which act as angiogenetic factors.

VEGF, also known as vascular permeability factor, is a secreted protein that may play a pivotal role in tumor-associated microvascular angiogenesis and hy-

perpermeability.[31,32] Kieser et al. demonstrated that the mutated p53 gene acts as a potent stimulator of VEGF.[5] It has also been shown that VEGF expression in colonic carcinomas correlates with IMVD and liver metastasis.[33] Recently, Kang et al. reported that p53 and VEGF staining status were identical in 65.6% of 163 colorectal carcinomas, with positive staining for both markers in 46 tumors and negative staining for both markers in 61 tumors.[34] They suggested the presence of a p53-VEGF pathway regulating tumor angiogenesis in human colorectal carcinomas. Our study did not detect a close correlation between p53 and VEGF expression. This is in good agreement with the report by Yamamoto et al., who examined 30 surgical specimens and 8 cultured cell lines derived from human gastric carcinomas and could not find a correlation between p53 gene status and VEGF expression.[35]

In contrast to VEGF expression, which was not affected by the TS treatment, the frequency of dThdPase expression was significantly higher in the p53 positive tumors than in the p53 negative tumors and was decreased by TS treatment. dThdPase is identical to platelet-derived endothelial cell growth factor (PD-ECGF) and has angiogenetic activity. Its expression has been shown to be higher in cancer tissues than in adjacent normal tissues and has also been shown to be associated with poorer prognosis, or with metastasis, in a variety of tumors, including breast, gastric, pancreatic, and colon carcinomas.[36,37] Although preoperative treatment with 5′-deoxy-5-fluorouridine (5′-DFUR), a prodrug of 5-FU, has been clearly demonstrated to reduce dThdPase activity in various cancer tissues, the relation between 5-FU or tegafur and expression of dThdPase has not yet been clearly elucidated. The current study suggests that the significant abatement of IMVD by TS treatment may have been caused by suppression of dThdPase expression, rather than by an effect on VEGF expression. Further study is needed to clarify the role of the p53 gene in the expression of VEGF and dThdPase.

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