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p53 Accumulation in Colorectal Cancer with Hepatic Metastasis

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The prevalence of immunoreactive p53 and argyrophilic nucleolar organizer region (AgNOR) numbers were compared between colorectal cancers with (n=44) and without (n=51) hepatic metastasis for at least 5 years. At the same time, the distribution of p53-positive cells in primary, metastatic, and xenografted tumors from the same individuals were studied. Overall, p53 positivity was found more frequently in the cases with hepatic metastasis than in non-metastatic controls, regardless of the distribution pattern ($P<0.05$), whereas AgNOR counts were not different between the two groups. Significant heterogeneity in the distribution of p53 immunoreactivity was noted in both the primary and metastatic lesions. The intratumor distribution patterns of p53 immunoreactive cells in the primary (n=33), metastatic (n=33), and xenografted (n=7) tumors of the same individuals were consistent in the majority of cases. There were a few cases in which the p53 immunoreactive cells were more dominant in the metastatic tumor cells. Our observations suggest that p53 accumulation in colorectal cancer is associated with increased risk for hepatic metastasis, while cell proliferation as represented by AgNOR numbers is not. In addition, heterogeneity of abnormal p53 accumulation in the tumor is maintained during the course of metastasis and even after implantation in nude mouse. p53-Immunoreactive cells in the population of colorectal cancer cells do not necessarily have higher metastasizing potential.

Key words: Colorectal cancer — Hepatic metastasis — p53 — AgNOR — Xenograft

Several biomarkers are known to represent increased risk of hepatic metastasis in colorectal cancer. The *p53* gene, which is the most frequently altered tumor suppressor gene so far studied in common human neoplasms,[1–3] is among the candidates. The association of *p53* mutation with prognosis in esophageal,[4] gastric,[5] and mammary[6] carcinomas has been reported. In colorectal cancer, some investigators have observed a correlation between *p53* mutation and metastasis or prognosis,[7–9] but others have not.[10–12] Furthermore, there are few reports on the intratumor distribution of p53-accumulating cells in primary and metastatic colorectal tumors from the same individuals and in xenografted tumors.

On the other hand, markers of cell kinetics such as Ki-67 and proliferative cell nuclear antigen (PCNA) have often been used as markers of the biological aggressiveness of various kinds of neoplasms.[13–15] The number of argyrophilic nucleolar organizer region (AgNOR) proteins, which are associated with ribosomal genes (rDNA) localized in the nucleolar organizer regions (NOR), is one such indicator. Although their exact biochemical nature is still controversial, they are probably regulatory proteins of rDNA transcription. Recently, the silver staining technique for the ultrastructural localization of AgNOR proteins was modified to a one-step method.[16] It has become a useful tool for the study of both the structure of the nucleus and variations in nucleolar activity. AgNOR counts were proposed to be a biological marker of cancer and its grade or prognosis in several cancers,[13, 14, 17–21] including colorectal. As to metastatic potential, Kakeji *et al.* recently reported a correlation between AgNOR numbers and lymph node metastasis of gastric cancer.[15] Therefore, it would be interesting to study whether putative biological aggressiveness as represented by AgNOR numbers contributes to the hepatic metastasis of colorectal cancer. As far as we know, there is no published information on the relation between AgNOR numbers and the metastatic potential of colorectal cancer.

The aim of this study was (1) to test the correlation of immunohistochemical accumulation of p53 tumor cells, numbers of AgNORs, and hepatic metastasis by comparing colorectal cancers with hepatic metastasis and those without cancer recurrence or distant metastasis for at least 5 years, and (2) to document the intratumor distribution of cells with p53 accumulation in the primary, metastatic and xenografted sites of the same patient's tumor, which might reflect in part the putative clonal expansion of p53-positive cells.

MATERIALS AND METHODS

Colorectal carcinoma with and without hepatic metastasis Ninety-five cases of advanced colorectal carcinoma

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were selected from the files of patients undergoing surgery from 1978 to 1993 in Hamamatsu University Hospital. These cases were divided into 44 cases of colorectal carcinoma with hepatic metastasis and 51 cases of non-metastatic colorectal carcinoma without recurrence or distant metastasis of carcinoma for at least 5 years after surgery. In the metastatic group, 10 of the 44 cases (24 male; 20 female) had metachronous hepatic metastasis with an interval of at least 12 months from resection of the primary tumor to hepatic metastasis. All the patients in the metastatic group underwent hepatic resection for metastatic tumors. When multiple hepatic metastases were resected in a patient, all the specimens were examined. Monthly measurement of serum carcinoembryonic antigen and regular examination by ultrasonography and computed tomography were used for follow-up study. Colorectal carcinoma associated with familial adenomatous polyposis was excluded.

Age, sex, location of the primary tumor, macroscopic type, size of tumor, histopathological grade of differentiation, lymph node metastasis and vessel involvement were tabulated and compared between the two groups (Table I). The locus, macroscopic type, depth of invasion, and histopathological differentiation of the primary tumors were classified according to the General Rules for Clinical and Pathological Studies on Cancer of Colon, Rectum and Anus.[22] Differentiation of the primary cancer was classified into two types: differentiated and non-differentiated. In comparing the clinical backgrounds of the two groups, the age of the metastatic group was found to be significantly younger than that of the non-metastatic group ($P<0.05$). In the metastatic group, the primary carcinoma was significantly more aggressive in terms of depth of cancer invasion ($P<0.05$), node metastasis ($P<0.001$), vein involvement ($P<0.0001$), and lymph vessel involvement ($P<0.01$) than in the non-metastatic group.

Xenograft In 7 cases, tumor tissues taken from metastatic cancer at surgery were implanted subcutaneously into nude mice, and passaged every 2 weeks until the subcutaneous tumor became enlarged enough to be palpable. Xenografted tumor was freshly taken at each passage for immunohistochemical and genetic analysis.

Antibodies Mouse monoclonal antibody DO7 for the detection of p53 protein was purchased from Novocastra Laboratories (Newcastle, UK). This antibody, which recognizes a denaturation-resistant epitope[23] of both the wild and mutant forms of human p53 protein, was diluted 1:100 with 0.01 mol/liter phosphate buffer saline (PBS) for use, according to the company's instructions. A Histofine kit (Nichirei Co. Ltd., Tokyo) including biotinylated anti-mouse immunoglobulin as the second antibody was used for the immunostaining of p53 protein.

Table I. Clinico-pathological Profile

	Metastatic group (n=44[a])	Non-metastatic group (n=51)
Age[b]	55.1	59.0
Sex (male:female)	24:20	24:27
Tumor site		
Right side	10	10
Left side	16	16
Rectum	18	25
Size		
<5 cm	14	24
≥ 5 cm	27	27
Macroscopic type[c]		
I	3	7
II	32	39
III	8	4
Other	0	1
Histologic grade		
Differentiated	41	45
Non-differentiated	3	6
Depth of invasion[b]		
mp[d]	1	7
ss, a_1[d]	20	30
s, a_2, si, ai_1[d]	22	14
Lymph node metastasis[e]		
Negative	12	34
Positive	32	17
Dukes' stage[e]		
A	1	7
B	11	27
C	32	17
Lymphatic invasion[f]		
Negative	20	40
Positive	24	11
Venous invasion[g]		
Negative	8	31
Positive	36	20

a) Data not available. Macroscopic type, 1 case; size, 3 cases; depth, 1 case.
b) $P<0.05$.
c) I, Protruding type; II, ulcerative and localized type; III, ulcerative and infiltrating type.
d) mp, muscularis propria; ss, subserosa; a, adventitia; s, serosa; si or ai, infiltration to other organs.
e) $P<0.001$. *f*) $P<0.01$. *g*) $P<0.0001$.

Immunohistochemistry Formalin-fixed, paraffin-embedded cancer tissues were used. Thinly sliced specimens of 3 μm were deparaffinized as usual. The antigen retrieval method as modified by our colleague[24] was applied in order to enhance the immunoreactivity of p53 protein in the paraffin sections. In brief, the sections were boiled in distilled water for 10 min by microwaving. Following pretreatment in methanol with 0.3% hydrogen peroxide for 15 min to inactivate endogenous peroxidase activity, the sections were rinsed with PBS and preincubated with

1% normal rabbit serum for 15 min at room temperature. Sections were incubated with the primary antibody (DO7) at room temperature for 1 h in a moist chamber, then with biotinylated anti-mouse immunoglobulin followed by peroxidase-conjugated streptavidin for 10 min at room temperature. Sections were rinsed with PBS three times, with 5 min between each step of incubation. Colorization was performed with 0.06 mmol/liter 3,3′-diaminobenzidine and 2 mmol/liter hydrogen peroxide in 0.05% Tris-HCl buffered at pH 7.6 for 10 min. Nuclear counterstaining was done with 1% methyl green. A tumor specimen with known *p53* mutation at codon 248 was used as a positive control. A section without the primary antibody was included in each experiment as a negative control.

Evaluation of immunoreactivity Distinct nuclear localization of the immunoprecipitate with monoclonal antibody DO7 was defined as positive, regardless of intensity. Actually, as far as our materials are concerned, immunoreactive p53 localization was restricted to nuclei, and cytoplasmic positivity was not identified. Semiquantitative evaluation of the numbers of positive cells and the area of positivity was performed on slides after antigen retrieval. The classification of the distribution pattern was based on examination of one to seven blocks covering the main portion of the tumor. The distribution of positive cells in each section was classified as sparse, focal, intermediate, or diffuse, using a standard light microscope. The numbers of cells with immunoreactive p53 per roughly 1000 cells were counted in three randomly selected high-power fields, and according to the average of these three evaluations, the distribution pattern of p53-positive cells was divided into four groups: diffuse, more than 70%; intermediate, 10–70%; sparse, less than 10%; and focal, aggregation of a few to ten positive cells. Representative pictures of these distributions are shown in Fig. 1.

AgNOR stain and counts According to Crocker and Nar,[25] deparaffinized sections were immersed in a solution containing 0.67% gelatin, 0.33% formic acid and

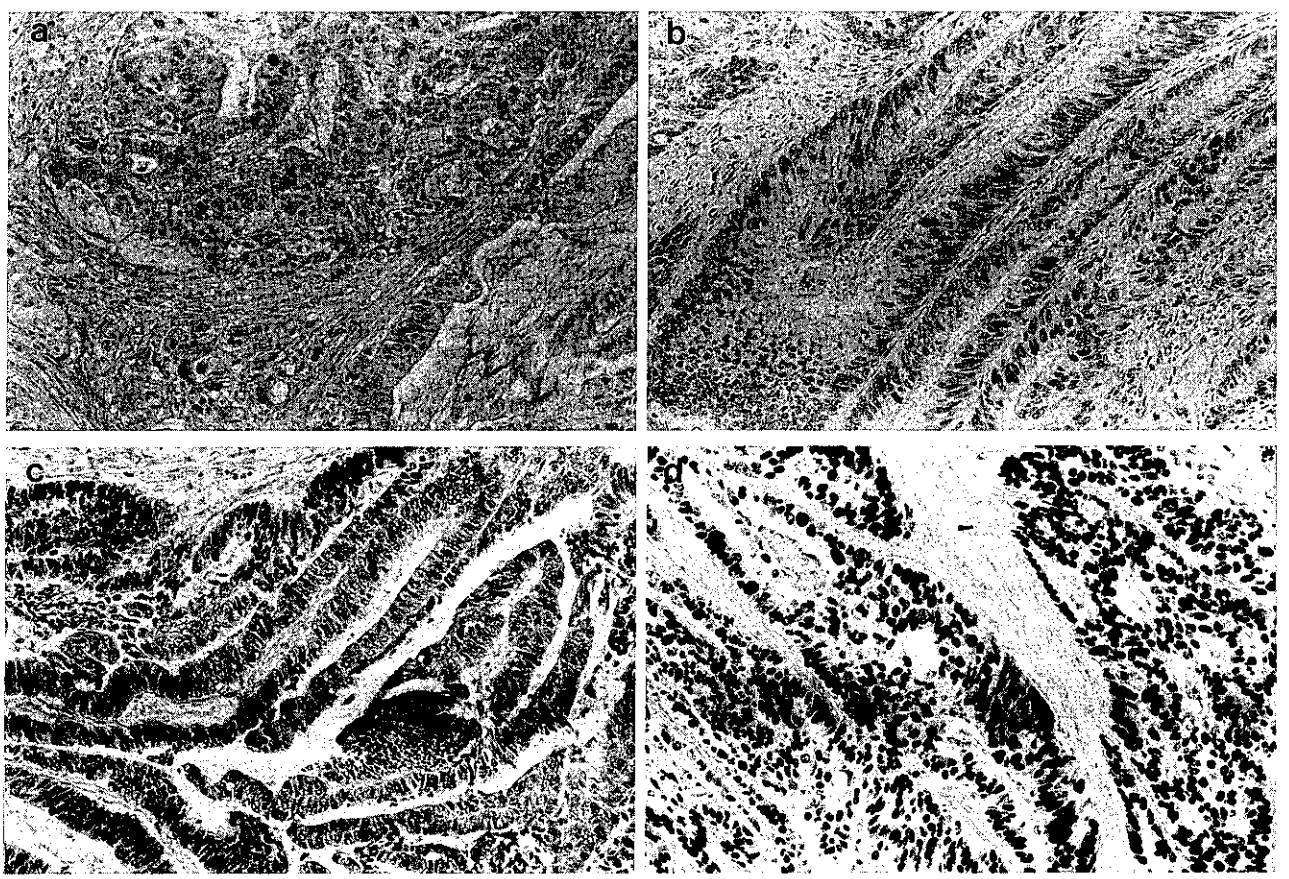

Fig. 1. Heterogeneity in the intratumor distribution of p53-positive cells. a) sparse, b) focal, c) localized (intermediate), and d) diffuse distribution of p53-positive cells. Streptavidin biotinylated complex stain, ×200.

33% aqueous silver nitrate for 20 min at 20°C in the dark. These sections were then treated with 5% sodium thiosulfate for 5 min, and the sections were slightly counterstained with 0.3% methyl green. The sections were observed with an oil immersion lens at a magnification of 1000, and intra- and extranucleolar brown or black dots with clear margins were counted within the nuclei of more than 100 cells of representative histological areas of colorectal cancer devoid of necrotic and necrobiotic changes and also within the nuclei of more than 100 non-neoplastic colorectal epithelial cells, respectively. The mean of the AgNOR numbers in these areas was calculated and defined as the AgNOR number of the case.

DNA analysis DNA extraction, primers for polymerase chain reaction (PCR) of *p53* exons 5 to 8, and single-strand conformation polymorphism (SSCP) analysis for screening *p53* mutation were described elsewhere.[26, 27]

Statistical analysis The chi-square test was performed for comparison of metastatic and non-metastatic groups for all clinicopathological features mentioned. Correlations between p53 positivity, AgNORs and clinicopathological features were also analyzed by means of the chi-square test. Statistical analysis for comparison of AgNOR numbers between two groups, and among p53-positive cases was performed using Student's *t* test. Multivariate analysis including major clinicopathological factors was performed in order to determine the factors related to hepatic metastasis. All the analyses were performed using standard computer software for statistical analysis (Excel Version 5.0, Microsoft).

RESULTS

p53 protein accumulation in primary tumors The immunoreactivity of p53 in colorectal cancer and hepatic metastasis in this series was dramatically enhanced by microwave treatment at 95°C for 10 min. Only cancer cells in the primary and metastatic tumors showed specific nuclear staining with monoclonal antibody DO7; normal colonic mucosa and liver tissue were completely negative for p53. Overall p53-positive rates in hepatic metastases with and without heat treatment were 82% and 36%, respectively. Therefore, p53 immunoreactivity is discussed on the basis of the results obtained with microwave treatment.

Table III. p53-Positivity of the Primary Lesion and Hepatic Metastasis

		Metastatic lesion of the liver		
		p53(+)	p53(−)	Total
Primary lesion	p53(+)	33[a)]	0	33
	p53(−)	0	11	11
	Total	33	11	44

a) One case was negative in the primary lesion, but the recurrent lesion showed a positive reaction.

Thirty-three cases (75.0%) in the metastatic group showed p53-positivity in the nuclei of cancer cells (Table II), compared with 26 cases (51.0%) in the non-metastatic group. p53 protein overexpression was observed at a significantly higher rate in the metastatic group ($P<0.05$). In the metastatic group, no significant difference in p53-positivity was found between the cases with synchronous metastasis and those with metachronous metastasis (Table II). Considering the distribution pattern of p53-positive cells, the prevalence of the diffuse distribution pattern was not significantly higher in the metastatic group (21/33 vs. 11/26, $P=0.17$). There was no significant correlation between p53 protein accumulation and any of the other clinicopathological factors, including depth ($P=0.3579$), node metastasis ($P=0.4137$), vein and lymph vessel involvement ($P=0.7566$ and $P=0.5876$, respectively). Multiregression analysis disclosed that p53, vascular invasion, and lymphatic invasion were strong predictors of hepatic metastasis, to the same extent (data not shown). These three factors seem to contribute independently to hepatic metastasis, or the numbers we analyzed were too small to allow detection of a mutual correlation.

Comparison of primary, metastatic and xenografted lesions in terms of intratumor immunoreactive p53 distribution In the metastatic group, all 33 cases with p53-

Table II. p53 Staining of Colorectal Cancer with and without Hepatic Metastasis Using Monoclonal Antibody DO7

	Metastatic group (n=44)				Non-metastatic group (n=51)	Total
	Synchronous metastasis	Metachronous metastasis	Total			
p53(+)	26	7	33 (75.0%)	]*	26 (51.0%)	59
p53(−)	8	3	11 (25.0%)	]	25 (49.0%)	36

* $P<0.05$.

Table IV. Distribution of p53-Positive Cells in the Primary Lesion and Hepatic Metastasis

	Metastatic tumor					Non-metastatic group
	Sparse	Focal	Intermediate	Diffuse	Total	
Primary Tumor						
Sparse	2		1		3	1
Focal	3	1			4	8
Intermediate		1	1	3	5	6
Diffuse		1	4[a)]	16	21	11
Total	5	3	6	19	33	26

a) One case was p53-negative in the primary lesion, but the recurrent lesion showed p53-positive.

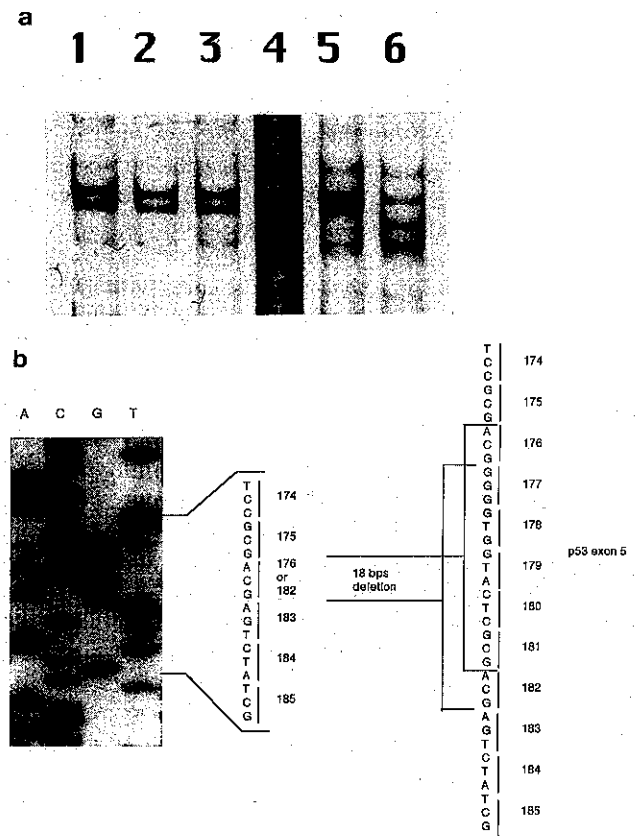

Fig. 2. SSCP analysis of DNA from the cases with sparse distribution pattern showed no abnormally migrated bands by SSCP analysis (a, lanes 1–5). The xenografted tumor which exhibited a diffuse pattern of immunoreactive p53 had an abnormal migrated band in SSCP analysis (a, lane 6) and an 18-base-pair deletion in *p53* exon 5 was demonstrated (b). This deletion was also detected in the primary tumor and the passaged tumor from the same patient (data not shown).

positive primary lesions showed p53-positive hepatic metastasis (Table III). One of the 33 cases with p53-positive hepatic metastasis had a p53-negative primary lesion, but the recurrent lesion in the rectum resected 5 years after the first operation was p53-positive, as was the metastatic lesion resected 8 months after the second operation. The 7 xenografted tumors did not show any alteration in p53 positivity at any passage. Five of 7 cases showed some p53 immunoreactivity in tumors, and 2 of the 5 had a sparse distribution.

In the primary tumor, heterogeneity was observed in the distribution of p53-positive cells, as shown in Fig. 1. The metastatic lesion showed the same distribution of p53-positive cells as the primary tumor in individuals. There was no correlation between the distribution pattern and clinicopathological parameters such as differentiation and size. In the metastatic group, among 20 of 33 cases with a p53-positive primary cancer, the distribution pattern of p53-positive cancer cells was unchanged between the primary lesion and hepatic metastasis. Sixteen of the 20 cases showed diffuse type in both the primary and metastatic lesions; 1 was intermediate, 1 focal and 2 sparse (Table IV). However, the p53-positive cells in 4 of the 33 cases were more expansive in hepatic metastasis than in the primary lesion. In 1 case, the sparse pattern in the primary lesion was increased to the intermediate type in the hepatic metastasis, and in 3, the pattern went from intermediate to diffuse. The tumor cells with immunoreactive p53 were less expansive in the metastatic lesion of the remaining 9 cases. The diffuse pattern in the primary lesion was reduced to the intermediate pattern in the hepatic metastasis in 4 of the 9 cases, from diffuse to focal in 1, from intermediate to focal in 1 and from focal to sparse in 3.

The original primary tumors and metastatic tumors had the same distribution pattern as the 7 xenografted cancers. *p53* mutations in exon 5, exon 7 and exon 8 were identified in 3 of the xenografted tumors and these cases had originally had diffuse p53 immunoreactivity (Fig. 2). Two of 7 xenografted tumors showed a sparse distribution of immunoreactive p53, and the profile of this intratumor distribution pattern was maintained during several passages (Fig. 3a–c). SSCP analysis for *p53* mutation was negative in those cases (data not shown). Two other xenografted cases which had no p53 accumulation

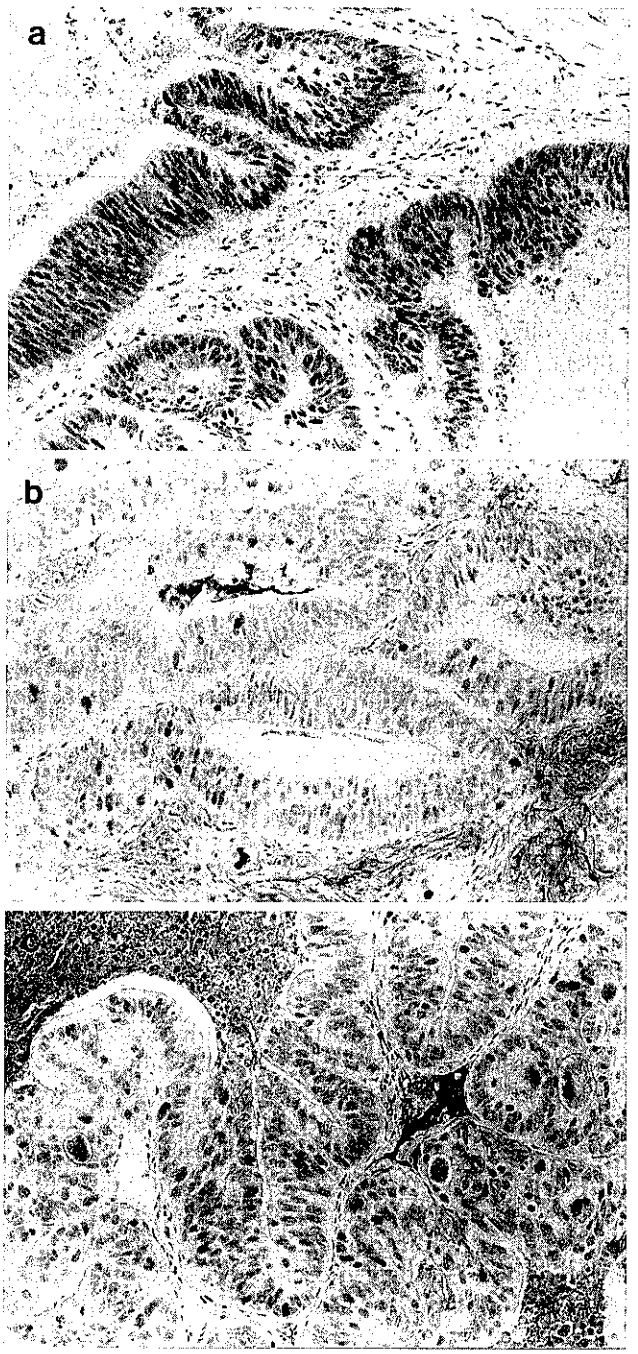

Fig. 3. Sparse distribution of p53-accumulating tumor cells in metastatic and xenografted tumors at different passages. a) hepatic metastatic lesion, b) passage 5, c) passage 9.

had no mutation from exons 5 to 8 of *p53*, either (data not shown).

AgNOR counts The mean of the AgNOR numbers (with SD) in non-neoplastic colorectal epithelial cells was 1.71 ±0.41 (65 cases; range 0.87–3.35). That in primary lesions of the hepatic metastatic group was 2.48±0.83 (22 cases; range 1.29–4.37), and that in primary lesions without metastasis was 2.54±0.63 (47 cases; range 1.44–4.27) (not significant). There was a significant difference in AgNOR numbers between total colorectal cancer (n=69) and normal mucosa ($P<0.001$), but there was no significant difference between the two groups (Table V).

Table V. AgNOR Numbers of Colorectal Cancer

Colorectal cancer (n=69)	2.52±0.69			*
Colorectal cancer with hepatic metastasis (n=22)	2.48±0.83	NS	**	
Colorectal cancer without hepatic metastasis (n=47)	2.54±0.63	*		
Normal colonic mucosa (n=65)	1.71±0.41			

NS: not significant.
* $P<0.001$. ** $P=0.008$.

p53 accumulation and AgNORs AgNOR numbers in the various degrees of p53 immunoreactivity were as follows: 2.64±0.85 in p53-abundant (diffuse type, n=20), 2.57±0.64 in the intermediate or sparse type (n=17) and 2.41 ±0.61 in p53-negative cases (n=32).

DISCUSSION

Our observations clearly showed that immunoreactive p53 in cancer cells is a concordant risk factor for hepatic metastasis, while AgNORs, a candidate biological grade and prognostic marker of malignancy, is not.

Many studies have examined p53 accumulation in cancer tissues.[28–30] Although we have kept in mind that there are some false-negative cases with nonsense mutations,[31] which occur in 20% of colon cancer, we analyzed the data based on p53 accumulation by immunohistochemistry, because we intended to estimate heterogeneous p53 accumulation in cancer tissue in primary, metastatic, and xenografted tumors. Furthermore, the antigen retrieval method with microwave treatment significantly influenced the distribution pattern and the prevalence of p53 immunoreactivity and enhanced the immunohistochemical detection of p53-positivity compared with the conventional method,[24] which many previous pathological studies had employed. However, since it is not certain whether the positivity represents the entire pool of particular antigens in the tissue, gene analysis such as PCR-SSCP and/or DNA sequencing will be required in future. Actual p53 sequences in the cases in which the immunohistochemical method could not detect the accumulation and their significance for metastasis are not known.

In human colorectal cancer, some investigators have reported a positive correlation of *p53* gene accumulation with prognosis,[8, 32] but others have not found this correlation.[10–12] Yamaguchi *et al.*[7] reported that the expression of p53 protein in colorectal cancer was positively related with short-term prognosis, but there was no correlation between *p53* gene expression and the pathological features of colorectal cancer. The same group reported that p53 overexpression in the endoscopic biopsy specimens of colorectal cancer showed positive relationships with recurrence and hepatic metastasis.[33] Iino *et al.*[10] reported that loss of heterozygosity (LOH) on chromosome 17p was significantly correlated with vascular invasion, and that allelic loss of 18q significantly correlated with hepatic metastasis. They also studied immunohistochemically the correlation between p53 overexpression and hepatic metastasis, but they did not demonstrate a statistically significant relationship between p53 accumulation and hepatic metastasis, partly because the number of cases was too small and/or they did not adopt the antigen retrieval method, which would influence the interpretation of p53 immunoreactivity. In our series, some clinical parameters such as lymphatic invasion and vascular invasion were more frequent in the metastatic group than in the non-metastatic control, but there was no significant correlation between p53 protein overexpression and any other clinicopathological features (Table I), including these stated above. This apparent discrepancy was probably because the number of tumors analyzed was too small to detect a correlation between p53 and vessel invasion.

On the other hand, AgNOR numbers did not differ between primary colorectal cancer without recurrence or metastasis and tumor cells with hepatic metastasis. This implies that the metastatic potential of colorectal cancer is independent of cell proliferative potential as represented by AgNORs and contradicts recent findings which showed that AgNOR numbers appeared to be related to poor prognosis of cancer of the endometrium,[17] esophagus,[18] kidney,[19] thymus,[20] stomach[14, 15] and breast.[13] A tendency for AgNOR numbers to increase according to semiquantitatively estimated p53 immunoreactivity was observed, and will require further investigation to understand its significance.

We attempted to examine whether or not the intratumor distribution pattern of p53 accumulation changes in primary cancer, hepatic metastasis, and xenografted tumors from the same patients. This was intended to study whether cancer clones with mutated (or accumulated) p53 in the primary tumor cell population could expand preferentially over the negative clones. Although the participation of p53 in clonal expansion of tumors was reported in brain tumors,[29] no information was given on the status of p53 in 2 (or more) chronologically different tumor stages in an individual patient.

We observed marked heterogeneity of p53 accumulation in primary, metastatic, and xenografted tumors. Our findings showed that this heterogeneity of p53 overexpression was common to colorectal cancer, and, contrary to our expectation, the p53-positive population of tumors did not seem to behave more expansively, although we have only immunohistochemical information and we do not know whether these small populations of p53 immunoreactive cells actually have mutant *p53* gene. The profile (presence or absence) of p53 immunoreactivity of hepatic metastasis was consistent with that of the primary lesion in all 44 metastatic cases, and furthermore, in the majority of cases, the distribution pattern of p53-immunoreactive cells in the hepatic metastases was also coincident with that of the primary lesion in the same individual. Very recently, Kastrinakis *et al.* reported that 6 cases out of 18 matching pairs had p53 staining only in the primary lesions.[34] Since the areas of the primary lesions that they immunohistochemically investigated are not described, we cannot discuss the possibility that a small population of *p53*-mutated cells might have expanded in the metastatic lesions. In two cases, the primary tumor contained very localized or sparse immunoreactive p53 cells, and its metastasis and xenografted tumor showed the same features of distribution. We did not detect *p53* mutations by PCR-SSCP and sequencing in such xenografted tumors with a sparse distribution of immunoreactive p53. Volkmann *et al.*[35] interpreted sparse distribution as heterogeneous control of *p53* expression in tumor cells or genomic heterogeneity in the tumor cell population. It is likely that the immunoreactivity to p53 antibody of the cells is not due to a *p53* mutation, but to heterogeneous control of *p53* expression in tumor cells that is conserved in the metastasis and xenografts in these cases. Anyway, our data at least do not support dominant proliferation of p53-immunoreactive clones in the majority of hepatic metastases of colorectal cancer, in contrast to the findings of Sidransky *et al.*[29] in primary brain tumor.

In one case, the primary tumor was p53-negative, while both the recurrent tumor and hepatic metastases were positive. An interpretation of this phenomenon would be that mutation of the *p53* gene occurred during the process of recurrence and that this *p53*-mutated clone became more expansive.

Ookawa *et al.*[36] examined restriction fragment length polymorphism markers encompassing 17p, 18q, 5q, and 13q in hepatic metastases of colorectal cancer and found that multiple genetic changes occur during the progression. They pointed out that LOH of 14q and 13q occurs more frequently in metastatic tumors and the prevalence of alteration involving 17p (LOH in this case) is not different between the primary and metastatic lesions. They examined both the primary and metastatic tumors

from the same patients in only 15 cases, and there was no information on the semiquantitative proportion of mutant tumor cells. Our data, together with theirs, suggest that there are additional genetic changes other than *p53* mutation which alter along with hepatic metastasis, while p53 accumulation in primary tumors is an increased risk factor for hepatic metastasis. It is still possible that cancer cells with *p53* mutations easily acquire metastatic potential, because such cells are genetically unstable, although the *p53* gene would not be directly involved in regulation of metastatic potential in colorectal cancer cells.

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