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p53 Immunohistochemistry of Gallbladder Carcinoma: A Comparison of PAb1801 and DO7 Antibodies

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Summary. **p53 gene mutations are common in many human cancers. Many investigators have examined cancer tissue by immunnohistochemistry using commercially available p53 antibodies. However, there have been only a few reports comparing different p53 antibodies. In this study, we have examined the distribution pattern, staining intensity and index of p53-positive cells with DO7 (DO-7) and PAb1801 (Ab-2) antibodies, using a 3- or 6-min diaminobenzidine (DAB) reaction time in 21 cases of gallbladder carcinoma to clarify which antibody is better for p53 detection. The distribution pattern of p53-positive cells was similar for both PAb1801 and DO7 in all but two cases. In staining with PAb1801, the p53-positive cell index was significantly higher at a 6-min reaction time than a 3-min reaction time (69.5±12.4% vs 85.1%±5.9%, $p<0.01$). In staining with DO7, the index was similar between 3-min and 6-min reaction times. There was no difference in the index between PAb1801 and DO7 for a 6-min reaction time (79.3±7.6% vs 85.1±5.9%). The staining intensity was stronger for 6 min than 3 min for both DO7 and PAb1801. Staining intensity at 6-min reaction time was similar between both antibodies. In summary, although PAb1801 staining is worse than DO7 staining for a 3-min reaction time, the two were similar with a 6-min reaction time. We conclude that the usefulness of PAb1801 is similar to DO7, provided a 6-min DAB reaction is used.**

INTRODUCTION

p53 gene mutations are common genetic abnormalities in many human cancers.[1,2] Analysis of the actual mutations is difficult and time consuming, and therefore is not routine in pathology laboratories. On the other hand, p53 itself is easily detectable in nuclei in many cancers by immunohistochemistry.[3-9] Two antibodies to p53, PAb1801 and DO7, are commonly used. Two previous reports concluded that the latter is more sensitive and specific than the former.[10,11] These reports compared the two antibodies by examining the relative proportions of p53-positive areas, but did not examine the intensity of staining for a given diaminobenzidine (DAB) reaction time or the proportion of p53 positive cells.

This study was intended to resolve the issue of which antibody is better for the detection of p53 overexpression, PAb1801 or DO7. We compared these antibodies by examining the intensity of staining and the p53 positive cell index in cases of gallbladder carcinoma.

MATERIALS AND METHODS

Twenty-one gallbladder carcinomas and four gallbladders with cholelithiasis, surgically resected between 1982 and 1993, were obtained from the archives of the First Department of Pathology at Niigata University. The carcinomas consisted of 10 early cases (mucosal cancer or cancer invading but not penetrating the proper muscle layer) and 11 advanced cases (cancer penetrating the proper muscle layer).

Six serial 3 μm-thick sections were made. A representative tissue block was made from each case, which had been fixed in 10% unbuffered formalin and embedded in paraffin. The first and fourth sections were stained with hematoxylin and eosin. The second and third sections were stained with p53-PAb1801 with a DAB reaction time of 3- and 6-min, respectively, and the fifth and sixth sections were stained with p53-DO7, with a DAB reaction time of 3- and 6-min, respectively.

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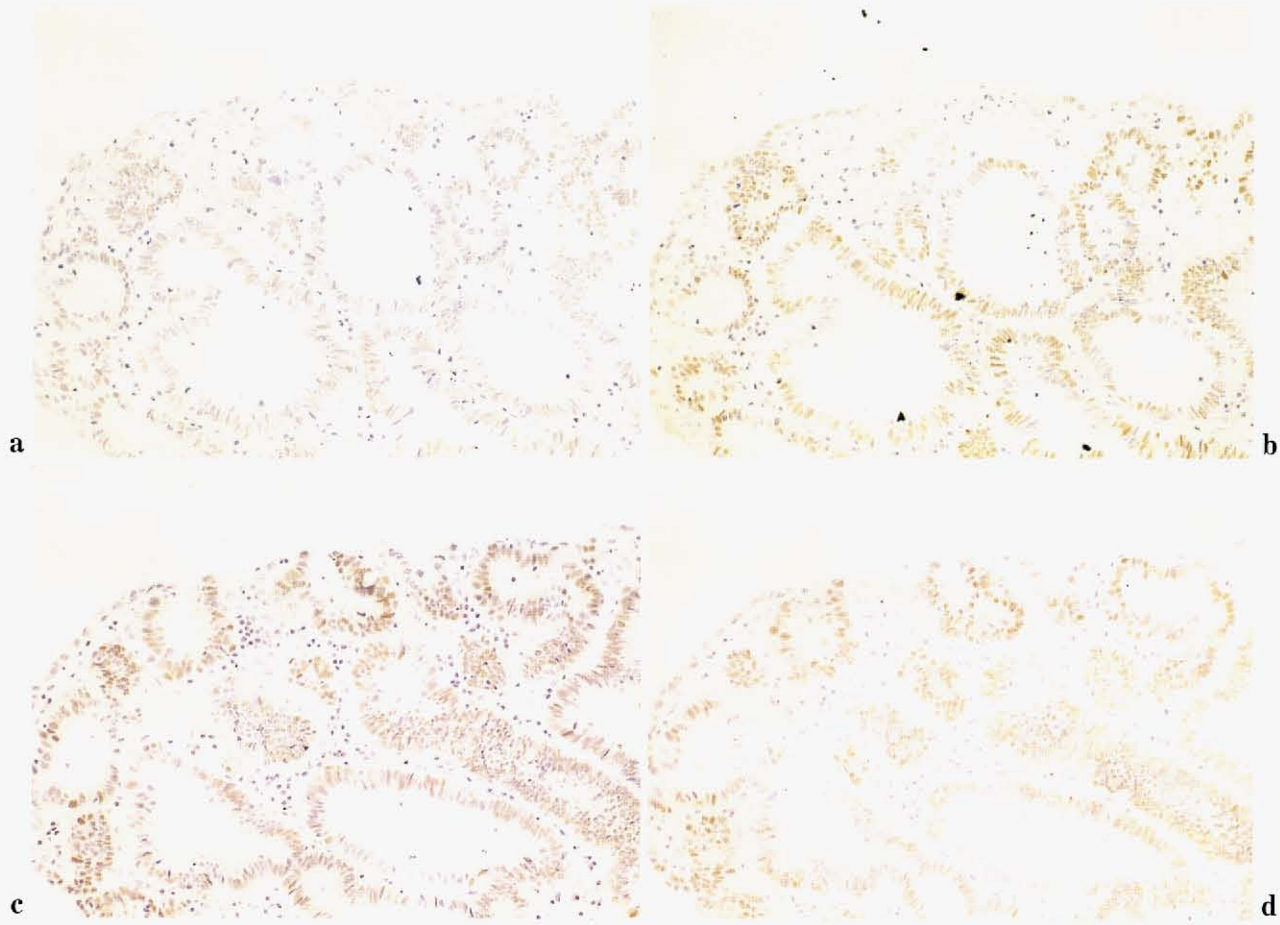

Fig. 1. Immunohistochemical staining with PAb1801 (**a, b**) and DO7 (**c, d**) staining at 3-min (**a, c**) and 6-min (**b, d**) DAB reaction times (Case no. 16). (**a**): weak nuclear staining at 3-min PAb1801 staining. (**b**), (**c**) and (**d**): diffuse and strong nuclear staining. ×100

For p53 immunostaining, paraffin-embedded sections were placed of poly L-lysine coated glass slides and air-dried at room temperature. Deparaffinized and rehydrated sections were heated in a microwave oven (500 W, Hitachi, MR-M220, Tokyo, Japan) for seven 3 min cycles in a citrate-buffer to retrieve antigenic activity, and then cooled for 60 min at room temperature.[12] Endogeneous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxidase in methanol for 20 min at room temperature. After blocking non-specific reactions with 10% normal rabbit serum, the sections were incubated first with human p53 monoclonal antibody DO7 (DO-7, Novocastra Laboratories Ltd., Newcastle, UK), which react with the 1–45 amino acid of p53 protein,[13] or PAb1801 (Ab-2, Oncogene Science Inc., Manhasset, NY, U.S.A.), which react with the 45–91 amino acid[13] at a dilution of 1 : 200 for 1 h. The sections then were incubated with biotinylated rabbit anti-mouse immunogloblin for 30 min and then with streptavidin-peroxidase complex (Histofine SAB-PO Kit, Biogenex Laboratories) for 15 min. Careful rinses were done with several changes of phosphate buffered saline between each step. The color was developed with diaminobenzidine; the sections then were lightly counterstained with hematoxylin and mounted.

Positive control sections were included in each experiment and consisted of tissue from a colonic adenocarcinoma with p53 positivity and a p53-mutation detected by PCR-SSCP (Polymerase chain reaction single strand conformation) and direct sequencing. Specimens from four cases of chronic cholecystitis were used in each experiment as negative controls.

p53-positive cells were defined as cells with brown nuclear staining, regardless of staining intensity. Intranuclear biotin inclusions with brown staining were not counted as p53-positive cells.[14] The pattern of p53-positive cells was classified as negative(−), sporadic(+),

Table 1. Comparison of p53 immunoreactivity between PAb1801 and DO7 antibodies

Case No.	Depth of invasion	Distribution pattern of p53 cells	PAb1801				DO7			
			Staining intensity		p53-positive cell index		Staining intensity		p53-positive cell index	
			3 min	6 min	3 min	6 min	3 min	6 min	3 min	6 min
1	EAR	−			0.0%	0.0%			0.0%	0.0%
2	EAR	−			0.0%	0.0%			0.0%	0.0%
3	EAR	−			0.0%	0.0%			0.0%	0.0%
4	EAR	−/+**			0.0%	0.0%		W	0.0%	0.0%
5	EAR	−/+**			0.0%	0.0%		W	0.0%	0.0%
6	EAR	+++	W	W	44.1%	77.4%	W	W	62.4%	76.3%
7	EAR	+++	W	Int	78.9%	79.0%	W	W	77.0%	77.8%
8	EAR	+++	W	Int	76.1%	81.5%	W	Int	73.4%	84.3%
9	EAR	++	Int	S	76.5%	89.6%	Int	Int	75.8%	87.9%
10	EAR	+++	S	S	68.2%	81.1%	S	S	78.6%	79.0%
11	ADV	−			0.0%	0.0%			0.0%	0.0%
12	ADV	−			0.0%	0.0%			0.0%	0.0%
13	ADV	−			0.0%	0.0%			0.0%	0.0%
14	ADV	−			0.0%	0.0%			0.0%	0.0%
15	ADV	−			0.0%	0.0%			0.0%	0.0%
16	ADV	+++	W	S	59.7%	88.6%	S	S	82.3%	85.6%
17	ADV	+++	W	S	52.2%	91.1%	S	S	89.4%	92.0%
18	ADV	++	Int	Int	67.6%	79.6%	Int	S	75.5%	82.4%
19	ADV	+++	Int	S	76.6%	85.1%	Int	S	84.4%	83.6%
20	ADV	++	S	S	75.8%	95.3%	Int	S	83.6%	93.2%
21	ADV	+++	S	S	88.5%	91.2%	S	S	90.3%	94.5%
					69.5±12.4%*	85.1±6.2%*			79.3%±7.6%*	85.1±5.9%*
					p<0.01				NS	
					p<0.01					
Chronic cholecystitis 4 cases		−			0.0%	0.0%			0.0%	0.0%
Positive control		+++	S	S	95.6%	95.5%	S	S	88.7%	92.8%

3 or 6 min=DAB reaction time of 3 or 6 min; ADV=Advanced carcinoma, EAR=Early carcinoma; −; negative, +; sporadic, ++; focal, +++; diffuse
W=weak, Int=intermediate, S=strong *: Mean±SD NS: p>0.05 **: PAb1801/DO7

focal (aggregations)(++) or diffuse(+++), and the latter two were designated as representing overexpression of p53-protein, as in our previous reports.[8,9]

Nuclear staining intensity was divided into three categories: weak, intermediate, and strong. The p53-positive cell index was expressed as the number of positive cells per 1,000 cells in the area counting the greatest number of positive cells in a homogeneous pattern. For p53 overexpression cases, differences between means were evaluated with Wilcoxon's rank sum test. Staining intensities and p53-positive cell indices were compared for corresponding areas of PAb1801 and DO7-stained sections.

RESULTS

The pattern of p53 expression was the same between PAb1801 and DO7-stained sections in all cases, regardless of the DAB reaction time, except for two cases (Nos. 4, 5) which did not show p53-protein overexpression (Table 1). The p53-positive cell index was significantly different between 3-min and 6-min DAB reaction times when stained with PAb1801 ($69.5\pm12.4\%$ vs $85.1\pm6.2\%$, respectively, $p<0.01$), but not between the two different staining times those with DO7 ($79.3\pm7.6\%$ vs $85.1\pm5.9\%$, respectively, $p>0.05$). There was, however, no significant difference in the index between 6-min staining with PAb1801 and 6-min staining with DO7.

The intensity of 6-min staining was either the same as or stronger than of 3-min staining for both antibodies (Fig. 1). The difference in intensity between 3 and 6 min was greater for staining with PAb1801 than with DO7. However, there was no major difference in the intensity of 6-min staining between DO7 and PAb1801.

DISCUSSION

In a previous study comparing commercially available p53 antibodies, including PAb1801 and DO7, Baas et al.[10] have concluded that DO7 staining has higher sensitivity, specificity, predictive value and efficiency in relation to the detection of p53-protein expression and p53 gene mutation, as compared with PAb1801 staining. They calculated the p53-labeling index by computerized image analysis which measured the total area of positive nuclear staining in selected regions of p53-stained sections, but did not allow the measurement of the number of positive cells. Moreover, they did not describe the DAB reaction time or the relationship between staining intensity and reaction time. Similarly, Lambkin et al.[11] have concluded that DO7 detects more p53 positive cases than PAb1801. However, they did not describe the staining time with PAb1801, the difference in staining intensity between DO7 and PAb1801, how the p53-positive cell index was calculated, or whether PAb1801 and DO7 stained sections were compared with each other in the corresponding areas.

In this study, the pattern of p53 staining was the same between 3- and 6-min reaction times for both PAb1801 and DO7 in most cases. In staining with PAb1801, the p53 positive cell index was significantly higher with a 6-min reaction time than a 3-min reaction time, while in staining with DO7, the index was similar between both reaction times. There was no difference in the index between both antibodies for a 6-min reaction time. Staining intensity with a 6-min reaction time was similar between both antibodies. From these results, a 6-min DAB reaction time seems to be necessary for sufficient PAb1801 staining. Lambkin et al.[11] have described a significant differnce in the detection of p53-protein overexpression between PAb1801 and DO7. Judging from our results, their data on PAb1801 staining seems to have been obtained using a shorter reaction time than 6 min. We conclude that the usefulness of PAb1801 is similar to DO7, provided a 6-min DAB reaction is used.

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