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Tanaka, Michiko
Watanabe, Makoto

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Comparison of Expression of *Griffonia simplicifolia* Agglutinin-1 and *Sophora japonica* Agglutinin Binding Sites in Relationship with p 53 Product in Sigmoid-rectal Polyps

Michiko Tanaka and Makoto Watanabe

Fifty-one specimens of sigmoid-rectal polyps were examined by lectin histochemistry and p 53 immunohistochemistry. *Griffonia simplicifolia* Agglutinin-1 (GSA-1) binding sites were recognized in 16 of 21 carcinomas and 6 of 30 adenomas. *Sophora japonica* Agglutinin (SJA) binding sites were recognized in 18 of 21 carcinomas, 1 of 19 adenomas adjacent to carcinoma and 16 of 30 adenoma. The p 53 expression were recognized in 15 of 21 carcinomas, 2 of 19 adenomas adjacent to carcinoma and 9 of 30 adenomas. Non-neoplastic mucosa were completely negative for GSA-1, SJA and p 53. The GSA-1 positive rate in carcinoma was similar to that of p 53. The SJA positive rate was higher than GSA-1 in each site of carcinoma, adenoma adjacent to carcinoma and adenoma. Moreover, we compared the staining results of GSA-1 and SJA with p 53 and GSA-1 with SJA. In carcinoma, the concordant rate of SJA and p 53 was higher, and in adenoma the concordant rate of GSA-1 and p 53 was higher. These findings showed that GSA-1 and SJA lectin histochemistry was a useful tool for the pathological diagnosis of the sigmoid-rectal polyps.

Key Words

Lectin histochemistry,
Griffonia simplicifolia,
Sophora japonica,
p 53 product.

Introduction

Alterations of the carbohydrate sequences were observed in many human cancers, and the distribution of specific carbohydrate sequences had been studied by lectin or anti-carbohydrate monoclonal antibodies. In our laboratory, lectin his-

tochemistry had been used to ascertain the distribution of glycoconjugates in sigmoid-rectal polyps¹⁻⁸⁾. Our previous data suggested that *Griffonia simplicifolia* Agglutinin-1 (GSA-1) binding sites appeared in carcinoma more frequently than on adenoma and non-neoplastic mucosa¹⁾. In this study, we performed lectin histochemistry using *Sophora japonica* Agglutinin (SJA) lectin, and compared it with GSA-1.

On the other hand, mutations of the tumor suppressor gene p 53, which is located on the human chromosome 17p had been found in high proportions in cancer tissues. The product of p 53 regulates normal cell growth. It is well known that the mutation of the p 53 gene leads to the accumulation of p 53

Faculty of Health Science, Kobe University
School of Medicine, Kobe, Japan.

product. Recently, the accumulation of p 53 product has been detected in many cancers by immunohistochemical procedures⁹⁻¹⁴.

In this study, we performed lectin histochemistry and p 53 immunohistochemistry for sigmo-rectal polyps and studied their relationships to carcinogenesis.

Material and Methods

Specimens

Fifty-one specimens of sigmo-rectal polyps were obtained by endoscopic polypectomy at Miki City Hospital (Miki, Japan) and were fixed with 10% buffered formalin. The specimens were embedded in paraffin and cut 4 µm thick. The tissue sections were stained with conventional hematoxylin and eosin (H.E.), and then used for lectin histochemistry and immunohistochemistry.

Lectins

Biotinated *Griffonia simplicifolia* Agglutinin-1 (GSA-1) and *Sophora japonica* Agglutinin (SJA) were purchased from EY Laboratory, San Mateo, CA. Lectin histochemistry was performed by the avidin biotin peroxidase complex (ABC) method (Vectastain Elite ABC Kit, Vector Laboratories Inc., Burlingame, CA).

Antibody

The anti-human p 53 protein antibody, from DAKO (Glostrup, Denmark), was used as the mouse monoclonal antibody. Immunohistochemistry was performed by a conventional LSAB Kit (DAKO Corporation, Carpinteria, CA).

Antigen retrieval

After blocking the activity of endogenous peroxidase, the sections were heated by a microwave oven (MR-A 330, Hitachi, Tokyo) for 15 minutes at 500 W, and then allowed to cool for approximately 30 minutes at room temperature²⁻³.

Staining methods

Lectin histochemistry was performed according to the previous published method¹⁻³. For p 53 immunohistochemistry, the sections were incubated with anti-p 53 protein for 30 minutes at room temperature, before applying the LSAB Kit.

For lectin histochemistry, the positive rate of the apical surface was checked. When one positive gland was recognized among other negative glands in a tissue section, that specimen was classified as positive. In p 53 immunohistochemistry, four immunostaining patterns; negative, sporadic, nested and diffuse, were identified. Negative and sporadic staining patterns were counted as negative, and nested and diffuse patterns were considered positive¹⁰.

Results

Histological diagnoses of the 51 specimens were indicated in Table 1. We compared each positive rate in accordance with the classification of cancer, adenoma and non-neoplastic mucosa, since the case numbers of each differentiated type of carcinoma and dysplastic grade of adenoma were rather small and unbalance. The positive rates of lectins and antibody binding sites in the polyps were shown in Table 2. GSA-1 binding sites were recognized in 16 of 21 carci-

Table 1. Histology of 51 polyps.

carcinoma (n=21)	well differentiated		18
	moderate differentiated		3
adenoma (n=30)	tubular	mild dysplasia	12
		moderate dysplasia	5
		severe dysplasia	2
	tubulovillous	mild dysplasia	2
		moderate dysplasia	8
		severe dysplasia	1
total			51

Table 2 a. Positive rates of lectins and anti p53 antibody binding sites in 21 carcinomas.

	GSA-1	SJA	p53
carcinoma	16/21(76%)	18/21(86%)	15/21(71%)
Adenoma adjacent to carcinoma	0/19	1/19(5%)	2/19(11%)
non-neoplastic mucosa adjacent to carcinoma	0/17	0/17	0/17

Table 2 b. Positive rates of lectins and anti p53 antibody binding sites in 30 adenomas.

	GSA-1	SJA	p53
Adenoma	6/30(20%)	16/30(53%)	9/30(30%)
non-neoplastic mucosa adjacent to adenoma	0/28	0/28	0/28

nomas and 6 of 30 adenomas. SJA binding sites were recognized in 18 of 21 carcinomas, 1 of 19 adenomas adjacent to carcinoma and 16 of 30 adenomas. The p53 expression were observed in 15 of 21 carcinomas, 2 of 19 adenomas adjacent to carcinoma and 9 of 30 adenomas. Non-neoplastic mucosa were completely negative for GSA-1, SJA and p53. A case of carcinoma stained by H. E., GSA-1, SJA and p53 respectively,

were shown in Figure 1. Concerning to the positive rates of GSA-1, SJA and p53, they were highest in carcinoma and SJA was highest among them, GSA-1 and p53 followed SJA. However the staining results of these three markers showed to be nearly the same, especially the GSA-1 and p53 positive rates. The positive rate for these three markers adenoma adjacent to carcinoma was very low comparing to adenoma. In adenoma

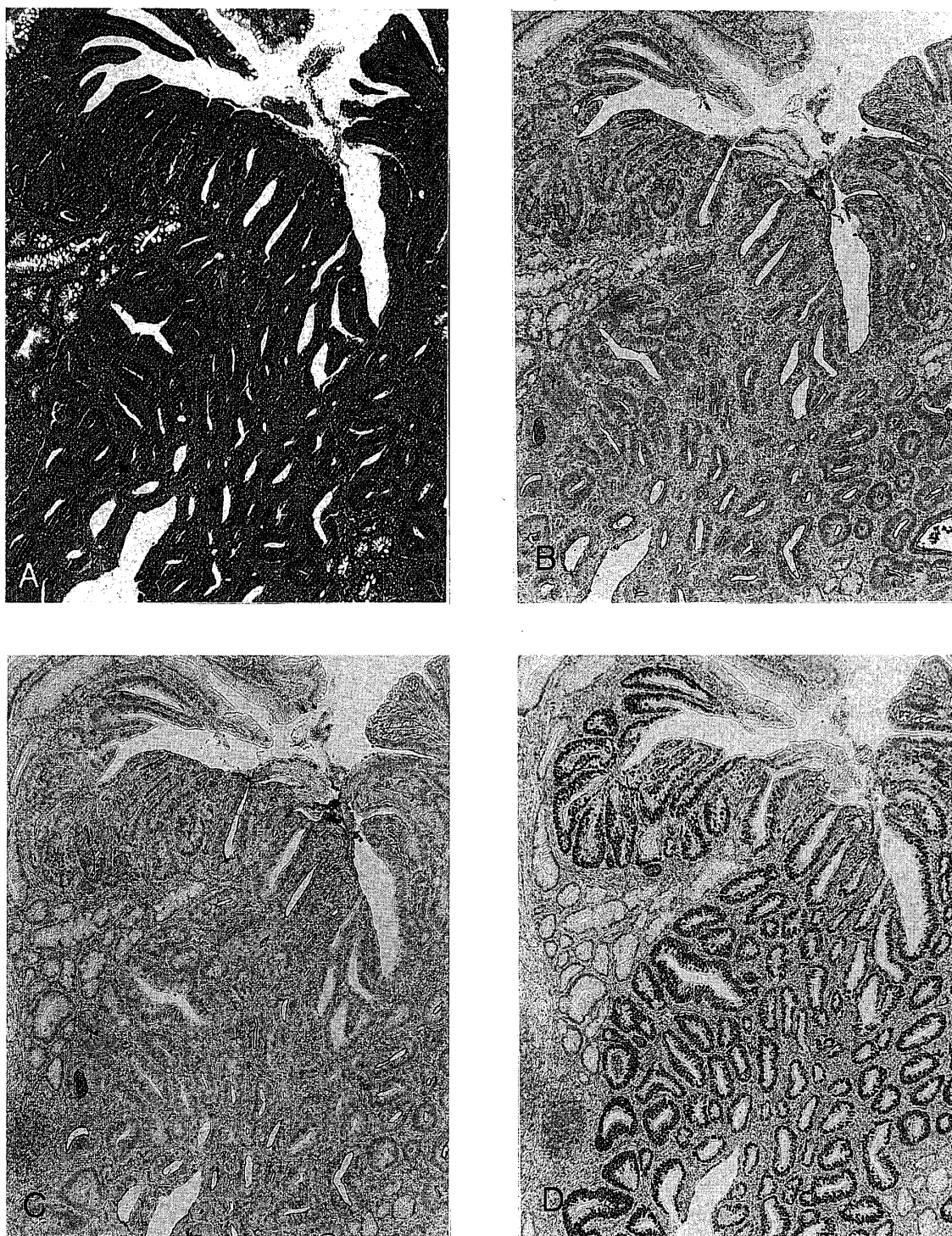


Figure 1. A case of carcinoma stained by (A) H.E., (B) GSA-1, (C) SJA and (D) p 53. GSA-1 and SJA binding sites were recognized on the apical surface in carcinoma. The p 53 products were recognized in the nuclei of carcinoma cells with a diffuse type pattern.

Table 3. Concordant rate of reagents.

	GSA-1 & p53	SJA & p53	GSA-1 & SJA
carcinoma	14/21(67%)	18/21(86%)	17/21(81%)
adenoma adjacent to carcinoma	17/19(89%)	16/19(84%)	18/19(95%)
adenoma	21/30(70%)	17/30(57%)	20/30(67%)
non-neoplastic mucosa	45/45(100%)*	45/45(100%)*	45/45(100%)*

* ; all negative

the positive rate of SJA was highest, p53 was next and GSA-1 was lowest. The SJA positive rate was higher than GSA-1 in carcinoma, adenoma adjacent to carcinoma and adenoma.

Moreover we compared the staining results of GSA-1 and SJA with p53 and GSA-1 with SJA (Table 3). The case numbers which revealed equivalent findings between GSA-1 and p53 were 14 of 21 in carcinoma, 17 of 19 in adenoma adjacent to carcinoma and 21 of 30 in adenoma. Those of SJA and p53 were 18 of 21 in carcinomas, 16 of 19 in adenoma adjacent to carcinoma and 17 of 30 in adenomas. In addition, those of GSA-1 and SJA were 17 of 21 in carcinomas, 18 of 19 in adenoma adjacent to carcinoma and 20 of 30 in adenomas.

Discussion

Recently, immunohistochemistry and various staining methods have been widely used as an auxiliary of the pathological diagnosis. We previously studied lectins which bind to carcinoma with a high frequency. Among them, we found that GSA-1 binding sites appeared on carcinoma more frequently than on adenoma and non-neoplastic mucosa. In this study, we observed that SJA had a

specificity similar to GSA-1 stained carcinoma and adenoma tissues, moreover, it did not stain non-neoplastic mucosa. However, SJA stained more heavily than GSA-1 in carcinoma and adenoma. It was considered that SJA recognized a greater number of sugar chains than GSA-1. When GSA-1 is positive, the probability of malignant change seems to be likely, but when negative, the judgment is difficult. Since SJA stained in a wider range than GSA-1, when SJA stains negative, it indicates that the possibility of malignant change is unlikely. In this study, we were unable to compare the positive rate of each differentiated type of carcinoma and dysplastic grade of adenoma, as each case number was too small and unbalance. That is, the number of well differentiated carcinoma was largest, 18 cases, while moderately differentiated carcinoma were only 3 cases. The number of each dysplastic grade of adenoma was usually less than 10 cases.

Additionally, we compared the staining result of these two lectins with p53 product. The expression of p53 was recognized in approximately 70% of the carcinoma. The p53 expression rate of colorectal carcinoma and adenoma were reported by many authors; 40 of 86 (46.5%) in carcinomas and 4 of 46

(8.7%) in adenomas (Purdie et al.)¹¹⁾, 7 of 11 (65%) in carcinomas, 15 of 36 (41%) in adenomas and none in non-neoplastic mucosa (Pignatelli et al.)¹²⁾, 50% in carcinomas and none in adenoma (Rodrigues et al.)¹³⁾, 51 of 84 (60.7%) in carcinoma, none in carcinoma adjacent to normal mucosa and 4 of 44 (9%) in adenomas (Kawasaki et al.)¹⁴⁾. When comparing their data with ours for carcinoma, our data was highest (71%). In addition, the positive rates of GSA-1 and SJA were higher than that of p 53. These findings together suggested that GSA-1 and SJA could be useful markers for pathological diagnosis in cancer and/or they would be more specific than p 53 in cancer diagnosis.

In this study, the positive rates of these three markers were lower in ade-

noma adjacent to carcinoma than in adenoma. As for the p 53 product, however, there were many nested types but few diffuse types in the positive pattern in adenoma. Pignatelli et al. noted that the genetic alterations of p 53 might be involved in the early steps of the adenoma-carcinoma sequence¹²⁾. Our data could support their report, but it is uncertain at present.

Comparing the concordant rate between GSA-1/p 53 and SJA/p 53, the concordant rate of SJA and p 53 in carcinoma was higher than that of GSA-1 and p 53, and the concordant rate of GSA-1 and p 53 in adenoma was higher than that of SJA and GSA-1. These data of concordant rate demonstrated that SJA had a grater staining ability than GSA-1.

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