

Clinicopathological study on pIgR expression and tumor progression in advanced colorectal cancer

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This study was performed in accordance with the Helsinki Declaration, and the Research Ethics Committee at Showa University Fujigaoka Hospital approved the protocol (Approval number; No. F2019C51).

Running title: Clinicopathological study of pIgR in colorectal cancer

Abstract

Objective. This study is aimed at investigating the relationship between the polymeric immunoglobulin receptor (pIgR) expression and clinicopathological factors in advanced colorectal cancer (CRC) patients. *Materials and Methods.* The study involved 47 advanced CRC patients who were surgically resected and underwent KRAS gene test. The pIgR expression was analyzed by immunohistochemistry, and the patients were classified into high and low (pIgR-H and pIgR-L, respectively) groups based on the staining intensity and range. *Results.* A total of 13 cases was classified under the pIgR-H group, and the remaining 34 were classified under the pIgR-L group. Results suggest no significant differences in most clinicopathological factors between the pIgR-H and pIgR-L groups, although the pIgR-L group had a significantly higher frequency of venous invasion than the pIgR-H group, whereas the frequency of KRAS gene mutation was significantly higher in the pIgR-H group than that in the pIgR-L group. *Conclusion.* The findings in this study showed little significant correlation between the pIgR expression and clinicopathological factors in advanced CRC

patients. Further research on the biological behavior of pIgR as a drug treatment option for KRAS-mutated advanced CRCs is also warranted.

Key words: polymeric immunoglobulin receptor, colorectal cancer, immunohistochemistry, clinicopathological study, KRAS

Introduction

Secretory IgA (SIgA) antibodies play a crucial role as the first line of antigen-specific immune defense, which protect the mucosal surfaces against environmental pathogens and antigens and maintain homeostasis with the commensal microbiota. The polymeric immunoglobulin receptor (pIgR) performs two roles: (1) transporting locally produced dimeric IgA across mucosal epithelia and (2) serving as the precursor of a secretory component, a glycoprotein that enhances the immune functions of SIgA. The complex regulation of pIgR expression and transcytosis by host and microbial factors is finely tuned to optimize the role of SIgA in mucosal immunity. Disrupting this regulatory network in disease states such as inflammatory bowel disease can adversely affect the mucosal homeostasis and systemic sequelae (1).

pIgR expression plays a role in carcinomas of various organs, such as the lung, colon, breast, endometrium, ovary, gallbladder, liver, pancreas, esophagus, and stomach, and its correlation with tumor development and prognosis has been considered (2-18). However, while Liu et al. (16) reported pIgR expression as a poor

prognostic factor for liver metastasis in colorectal cancer (CRC), the significance of the pIgR expression in CRC remains unknown.

This study is aimed at determining the correlation between the pIgR expression and clinicopathological factors in CRC patients especially those requiring adjuvant treatment after surgical resection, with the aim of developing a clinical solution to KRAS-mutant-type CRC.

Materials and methods

The study pooled 389 advanced CRCs surgically resected from January 2016 to December 2018 at Showa University Fujigaoka Hospital and evaluated a final sum of 47 patients who had been histopathologically diagnosed with ordinary CRC (tubular adenocarcinoma) and undergone a KRAS mutation test due to the need for adjuvant treatment after surgical resection. The extracted specimens included cases with no obvious lymph node metastasis (N0). Cases with no pathological lymph node metastases, due to distant metastases or other factors, were still considered to be at high risk, and postoperative chemotherapy was done.

The immunohistological analysis of the pIgR expression in CRC tissues was performed using the avidin–biotin complex detection method with standard protocols employing a Leica Bond system (21). Briefly, formalin-fixed, paraffin-embedded tissue sections were pretreated using heat-mediated antigen retrieval with sodium citrate buffer for 20 min. Sections were then incubated with primary antibodies against pIgR (1:500 dilution; no. ab96196; Abcam, Cambridge, UK) for 15 min. at room temperature and were detected using a horse radish peroxidase-

conjugated compact polymer system and DAB as the chromogen. Sections were then counterstained with hematoxylin.

Two authors of this study (K.K. and N.O.) who were blinded to the clinical parameters separately reviewed and scored the immunostained tissue sections. The degree of immunostaining was based on the intensity of staining and percentage of cells stained. Staining intensity was graded accordingly as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. Moreover, staining percentages were graded according to the proportion of positively stained tumor cells as follows: 1 for <30% positive tumor cells; 2 for 30%–70% positive tumor cells; and 3 for >70% positive tumor cells. The pIgR expression was evaluated based on the staining index (score: 1 to 6). As an arbitrary but optimal cut-off value, we defined a total staining index score of ≤ 4 to indicate a low pIgR expression (pIgR-L), while an index score of ≥ 5 to indicate a high pIgR expression (pIgR-H) (Figure 1).

A clinical testing company (Bio Medical Laboratory, Inc., Kawagoe, Japan) analyzed KRAS gene mutations using the PCR-rSSO method.

Clinicopathological comparisons between the pIgR-H and pIgR-L groups and the KRAS-mutant and KRAS-wild-type groups were performed using several

factors described in the Japanese Classification of Colorectal, Appendiceal, and anal carcinoma (22).

Statistical analyses were carried out using Student's *t*-test and Welch's *t*-test (JMP software program, version 14; SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Results

Immunostaining, scoring, and grouping of pIgR

The background normal mucosa showed the homogeneous diffuse expression of pIgR in the cell membrane and cytoplasm of the glands, whereas tumor cells showed various staining patterns within and between tumors. Table 1 details the scores of the 47 cases in this study: 13 cases in the pIgR-H group and the remaining 34 cases in the pIgR-L group.

Clinicopathological comparison between the pIgR-H and pIgR-L groups

Among the factors between the pIgR-H and pIgR-L groups, most factors (except for venous invasion and KRAS mutation), such as the sex, mean age, location, macroscopic type, mean tumor size, circumference ratio, histological type,

infiltration pattern, depth of invasion, lymphatic invasion, perineural invasion, lymph node metastasis, and mean survival period, showed no significant differences (Table 2). A significantly higher frequency of venous invasion was found in the pIgR-L group compared to that in the pIgR-H group, whereas the frequency of KRAS gene mutation was significantly higher in the pIgR-H group (77%, 10/13) than that in the pIgR-L group (44%, 15/34).

Clinicopathological comparison between the KRAS-mutant and KRAS-wild-type groups

KRAS mutations were detected in 25 of 47 cases. The types of mutations were as follows: A146T, 1case; G12A, 2; G12C, 1; G12D, 12; G12V, 2; G13D, and 5; Q61H, 1.

Furthermore, between the KRAS-mutant and KRAS-wild-type groups, most factors (except for the mean age and pIgR expression), such as the sex, location, macroscopic type, mean tumor size, circumference ratio, histological type, infiltration pattern, depth of invasion, lymphatic invasion, venous invasion, perineural invasion, lymph node metastasis, and mean survival period, showed no

significant differences (Table 3). The KRAS-mutant group consisted of significantly older subjects, and a high pIgR expression was observed to be significantly more frequent in the KRAS-mutant group (40%, 10/25) than that in the KRAS-wild-type group (13%, 3/22).

Discussion

The mucosal surface of the gastrointestinal tract is overwhelmed with various stimuli. Protection from these stimuli is important, and innate and adaptive immunities must function cooperatively. The surfaces of mucosal sites are covered by epithelial cells, such as the intestinal epithelial cells (IECs). IECs form a physical barrier and drive innate and adaptive immunity against invading pathogens to maintain intestinal homeostasis. The most important adaptive immune system in mucosal sites is the mucosal immune system, and the main player in this system is the polymeric immunoglobulins (pIgs), which are produced by antibody-secreting plasma—cells that accumulate in the lamina propria. In order to exert a protective function, pIgs are transported to the luminal side, across IECs, then finally released into the intestinal lumen. This process is called transcytosis and is mediated by a glycoprotein, pIgR (9).

While the abnormal expression of pIgR is generally known in malignant tumors, the clinical relevance and potential function of pIgR in the tumor cells remain to be topics to be explored. Studies on the relationship between the pIgR expression and malignant behaviors yield varying findings across cancers.

Moreover, concerning studies on gastrointestinal and hepatobiliary-pancreatic cancers, Ai et al. showed that a high expression of pIgR was significantly associated with early recurrence in early-stage hepatocellular carcinoma (HCC) and hepatitis B surface antigen-positive HCC patients (8), whereas Richard et al. conversely reported that a high pIgR expression independently predicted a decreased risk of recurrence and an improved survival in patients with adenocarcinoma of the upper gastrointestinal tract (10). In addition, a high tumor-specific pIgR expression showed a more favorable tumor phenotype, while a low expression independently predicted a shorter survival in patients with pancreatic and periampullary cancer (11).

As revealed in the study of Liu et al. (16), a positive expression of pIgR was significantly associated with a poor prognosis in colon carcinoma patients with hepatic metastasis. In our study, however, significant differences on clinicopathological examinations between the pIgR-H and pIgR-L groups were found in only a few factors (venous invasion and KRAS mutation). Due to the small number of cases, there is little certainty to deduce that pIgR expression cannot be a significant prognostic factor.

Patients with gene mutations are considered “mutant type,” whereas those without are considered “wild type.” KRAS mutations have emerged as aggressive drivers of disease. However, while it has been more than 30 years since the discovery of the role of KRAS in transforming cells and driving cancer progression, there are still currently no pharmaceutical breakthroughs that are able to address the activating mutations of KRAS or to selectively down-regulate KRAS mRNA and proteins, nor are there any inhibiting downstream effector pathways in clinical trials. This elevates the problem we already face in treating the three most deadly cancers—pancreatic cancer, non-small-cell lung cancer, and CRC—as KRAS mutations are associated with a poor prognosis in these tumor histotypes (23).

Moreover, a significant correlation was found between KRAS mutations and the pIgR expression, which no previous research was able to reveal (9). The molecular mechanism underlying the correlation is yet unknown, and perhaps a coincidence; nevertheless, the development of pIgR-targeted therapeutics is expected to be useful in treating KRAS-mutant-type CRCs.

Conclusions

The findings in this study reveal that there is little significant correlation between pIgR expression and clinicopathological factors in patients with advanced CRC. Further accumulation of cases and careful examination are required whether studies focusing on pIgR as a treatment strategy for KRAS-mutant-type CRC may be meaningful or not.

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Conflict of Interest: None

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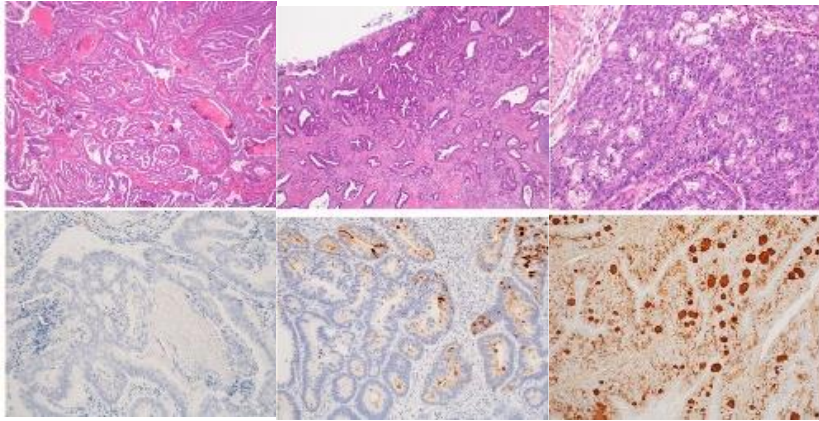


Figure 1. Hematoxylin & eosin staining (upper) and immunohistochemical staining of PIGR (lower) of colorectal cancers. The left panel had a score of 2 and the middle panel a score of 4, both of which were classified into the pIgR-low group, while the panel on the right had a score of 6 and was classified into the pIgR-high group.

Table 1. Patient characteristics

N=47					
Age	year, mean	67.3			
Sex	male:female	30(63.8%):17(36.2%)			
Tumor location	right:left	14(29.8%):33(70.2%)			
Macroscopic type	1:2:3:4:5	3(6.4%):25(53.2%):16(34%):1(2.1%):2(4.3%)			
Tumor diameter	mm, mean	64.4			
Circumference ratio	%, mean	85			
Histological type	adenocarcinoma (well differentiated: moderately differentiated)	15(31.9%):32(68.1%)			
Infiltration pattern	expansive : intermediate : infiltrative	18(38.3%):7(14.9%):22(46.8%)			
Depth of tumor invasion	T2:T3:T4	2(4.3%):30(63.8%):15(31.9%)			
Lymphatic invasion	none:minimal:moderate:severe	5(10.6%):33(70.2%):6(12.8%):3(6.4%)			
Venous invasion	none:minimal:moderate:severe	0(0%):12(25.5%):28(59.6%):7(14.9%)			
Perineural invasion	none:intramural only: extramural	16(34.0%):17(36.2%):14(29.8%)			
Lymph node metastasis	N0:N1:N2:N3	15(31.9%):14(29.8%):10(21.3%):8(17.0%)			
Survival period	month, mean	28.6			
pIgR	score	1	0(0%)	low	34(72.3%)
		2	16(34.0%)		
		3	8(17.0%)		
		4	10(21.3%)	high	13(27.7%)
		5	8(17.0%)		
		6	5(10.6%)		
RAS	mutant:wild	25(53.2%):22(46.8%)			

Table 2. Clinicopathological comparisons between the pIgR-H and pIgR-L groups

Group	pIgR-H group (13 case)	pIgR-L group (34 case)	<i>p</i> -value
Sex (Male:Female)	7:6	23:11	NS
Age (mean, years)	66	68	NS
Tumor location (Right:Left)	4:9	10:24	NS
Macroscopic type (1:2:3:4:5)	1:8:3:0:1	2:17:13:1:1	NS
Tumor diameter (mean, mm)	67.2	63.3	NS
Circumference ratio (mean, %)	89	83	NS
Histological type, Adenocarcinoma (well differentiated : moderately differentiated)	6:7	9:25	NS
Infiltration pattern (expansive : intermediate : infiltrative)	5:2:6	13:5:16	NS
Depth of tumor invasion (T2:T3:T4)	2:4:7	0:26:8	NS
Lymphatic invasion (none:minimal:moderate:severe)	1:10:2:0	4:23:4:3	NS
Venous invasion (none:minimal:moderate:severe)	0:6:7:0	0:6:21:7	p=0.02
Perineural invasion (none:intramural only:extramural)	5:5:3	11:12:11	NS
Lymph node metastasis (N0:N1:N2:N3)	5:5:0:3	10:9:10:5	NS
Survival period (month, mean)	23 (n=12)	18 (n=27)	NS
RAS gene (mutant:wild)	10:3	15:19	p=0.04

NS: not significant

Table 3. Clinicopathological comparisons between the KRAS-mutant and KRAS-wild-type groups

Group	KRAS-Mutant type (n=25)	KRAS-Wild type (n=22)	<i>p</i> -value
Sex (male:female)	16:9	14:8	NS
Age (mean, years)	70	64	0.04
Tumor location (right:left)	10:15	4:18	NS
Macroscopic type (1:2:3:4:5)	3:13:8:0:1	0:12:8:1:1	NS
Tumor diameter (mean, mm)	63	66	NS
Circumference ratio (mean, %)	86%	83%	NS
Histological type, Adenocarcinoma (well differentiated : moderately differentiated)	11:14	4:18	NS
Infiltration pattern (expansive : intermediate : infiltrative)	0:22:3	0:18:4	NS
Depth of tumor invasion (T2:T3:T4)	2:15:8	0:15:7	NS
Lymphatic invasion (none:minimal:moderate:severe)	3:20:2:0	2:13:4:3	NS
Venous invasion (none:minimal:moderate:severe)	0:7:16:2	0:5:12:5	NS
Perineural invasion (none:intramural only:extramural)	8:8:9	8:9:5	NS
Lymph node metastasis (N0:N1:N2:N3)	10:6:6:3	5:8:4:5	NS
Survival period	28 (n=24)	29 (n=18)	NS
pIgR expression (high:low)	10:15	3:19	0.04

NS: not significant.