International Journal of Surgery 12 (2014) 815–820

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

HLA-Cw polypmorphism and killer cell immunoglobulin-like receptor (KIR) gene analysis in Korean colorectal cancer patients

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ARTICLE INFO

Article history:
Received 27 March 2014
Received in revised form
3 June 2014
Accepted 22 June 2014
Available online 4 July 2014

Keywords:
Colorectal cancer
KIR
HLA-Cw
NK cells

ABSTRACT

Purpose: Natural killer cells (NK cells) play important roles in protecting the patient from the early development of cancers, and are activated or inhibited by killer cell immunoglobulin-like receptors (KIR), which bind to HLA class I. In the present study, we investigated the KIR genotype of Korean colorectal cancer patients.

Methods: DNA samples were extracted from peripheral blood cell samples taken from Korean colorectal cancer patients and a control group. KIR genes were amplified using PCR-SSP methods, and HLA-Cw genes were characterized using PCR methods. The results were analyzed to assess the difference between colorectal cancer patients and the normal control group.

Results: In the present study, the frequency of KIR2DS5 (33.2% vs. 20.8%, p-value < 0.007) was higher in the colorectal cancer group, and in the rectal cancer subgroup, the frequencies of KIR3DL1 (93.2%, vs. 98.1%, p-value < 0.05), KIR2DS2 (7.8% vs. 19.5%, p-value < 0.01), and KIR2DS4 (93.2% vs. 98.1%, p-value < 0.05) were lower significantly. The frequencies of HLA-Cw6 (9.1% vs. 15.7%, p-value < 0.05) and HLA-Cw7 (17.4% vs. 27.7%, p-value < 0.02) were lower in the colorectal cancer group. Of the patients with HLA-C1 homozygote, the frequency of KIR2DS2 was decreased significantly (5.8% vs. 14.5%, p-value < 0.004).

Conclusions: The frequency of KIR2DS5 is higher in Korean colorectal cancer patients, and in the rectal cancer subgroup, the frequencies of KIR3DL1, KIR2DS2 and KIR2DS4 are lower. Among the patients with HLA-C1 homozygote, the frequency of KIR2DS2 is decreased. Therefore, KIR2DS2 in presence of its ligand (HLA-C1 group) may have a protective effect against colorectal cancer.

1. Introduction

Colorectal cancer is the one of the most common incident cancer worldwide [1]. In Korea, incidence of colorectal cancer has increased annually. And colorectal cancer has become the second most common cancer after stomach cancer among men and the third most common cancer after cancers of the thyroid and breast among women in Korea [2].

A wide range of genetic and environmental factors are involved in the development and progression of colorectal cancer. The immune system of the patient also plays an important role during early cancer cell elimination and protection against metastasis. Overcoming the patient's immune system is critical to tumor growth. Of the various elements of a patient's immune system, the innate immunity is responsible for the first protection against the cancer cells [3].

Natural killer cells (NK cells), which constitute about 10–15% of the peripheral lymphocytes, are among the most important of the innate immune cells, and can destroy cancer cells without previous sensitization [3,4].

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http://dx.doi.org/10.1016/j.ijsu.2014.06.012
1743-9191/

There are many receptors on the surface of human NK cells. Of these, killer cell immunoglobulin-like receptors (KIR, CD158), which recognize human leukocyte antigen (HLA) class I as their ligands, can activate or inhibit NK cells plays an important role in controlling the function of NK cells [5]. KIR types are classified into an inhibitory group with a long (L) intracytoplasmic tail and an activating group with a short (S) intracytoplasmic tail. The genetic distribution of KIR genes effects the expression of KIR on NK cells, though ethnic differences in KIR genotypes have been observed [6]. KIR genes are encoded on chromosome 19q13.4. The KIR gene cluster comprises up to 16 highly homologous and closely linked genes and pseudogenes [7]. And there are 7 kinds of inhibiting genes and another 7 kinds of activating genes [8].

The ligands of KIR are HLA class I (A, B, C), and most previous studies have been focused on the HLA-C alleles. HLA-C alleles can be divided into the HLA-C1 group that carries an asparagines residue at position 80 and HLA-C2 group that has a lysine residue at position 80 [9]. It is known that KIR2DL1 and KIR2DS1 bind to HLA-C2, and KIR2DL3, KIR2DS2, and KIR2DL2 bind to HLA-C1 [10]. Specificities of the remaining KIRs are not as well characterized thus far.

The inhibiting KIRs recognize the self HLA I as a ligand, and play an important role in maintaining self tolerance by inhibiting NK cells [11]. In other words, the cells that do not express self HLA I will be destroyed by activated NK cells. Cancer can develop by overcoming the host immune systems in a number of ways. One of the mechanisms is decreasing the expression of HLA on cancer cells. Through the decreased expression of HLA on the cell's surface, delivery of the cancer antigen to T lymphocytes is decreased [12]. But the lack of expression of HLA on the cancer cells also may decrease the inhibiting function of inhibiting KIR on NK cells.

There have been several reports concerning the relationship of KIR genes and disease, including autoimmune disease, infectious disease, transplantation, stem cell disease, and malignant diseases [10,13]. In terms of the malignant diseases, there have been studies of colon cancer, renal cancer, lung cancer, laryngeal cancer, malignant melanoma, and cervical cancer [14–18]. However, two other studies on colon cancer did not find any differences in KIR genes between the cancer group and the normal control group [15,16].

In the present study, we compared the HLA-Cw polymorphisms and KIR genotypes between the colorectal cancer patients and normal control group in Korean populations. We were trying to investigate the characteristic of KIR genotypes and HLA-Cw polymorphisms in Korean colorectal cancer patients, and the bio-immunological role of KIR genes in the development of the colorectal cancer.

2. Materials and methods

2.1. Study group and control group

This study was performed using the peripheral blood of Korean colorectal cancer patients and a normal control group from the general Korean population. Of the colorectal cancer patients who underwent surgery between March, 2007 and December 2008, blood samples from 241 (60%, 241/399) patents' were used for this study. The control group was made up of 159 persons who were healthy and had no disease and cancer history [19,20]. This study was approved by the Institutional Review Board of the Catholic Medical Center of The Catholic University of Korea (KC10TISI0382).

2.2. DNA extractions

Extractions of the DNA were performed using the AccuPrep Genomic DNA Extraction kit (Bioneer corporation, Daejeon, Korea). In brief, the lymphocytes were separated from 1 ml of peripheral blood using RBC lysis buffer (1 M Tris–HCl. pH 7.6, 1 M KCl, 0.5 M $MgCl_2$, 0.5 M EDTA, 2.5% N-P40). The separated lymphocytes were suspended with PBS 200 μl, and reacted with 20 μl of proteinase K (20 μg/ml), and 200 μl of binding buffer (GC) for 10 min at 60 °C, and were then transferred to bind column with 100 μl of isopropanol. Washings were performed with washing buffer (W1, W2). DNA was extracted through centrifugation with the washed column and 200 μl of distilled water. The concentrations of extracted DNA were checked using spectrophotometer, and used for PCR at a concentration of 100 ng/μl.

2.3. KIR genotyping

The KIR genes were determined by polymerase chain reaction using sequence-specific primers (PCR-SSP) [14]. Inhibitory KIR were typed for KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, and KIR3DL3, activating KIRs were typed for KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5 and KIR3DS1, and pseudo KIRs were typed for KIR2DP1 and KIR3DP1 [21]. Specific amplifications of the KIR genes were performed using 50 forward and reverse primers. PCR was carried out with primers in a reaction volume of 10 μl containing 1× buffer (50 mM KCl, 10 mM Tris–HCl pH 9.0, 1.5 mM $MgCl_2$, 0.1% Triton X-100), 0.1–0.5 μM of each nucleotide primer, 2.5 mM dNTPs, 100 ng genomic DNA and 0.25 U I-start *Taq* Polymerase (iNtRON biotechnology, Seongnam, Korea). The amplifications were carried out in a My Cycler™ thermocycler (Bio-Rad, Hercules, CA, USA). A total of 35 cycles of PCR were completed using the following steps: 25 s at 91 °C, 45 s at 65 °C, 30 s at 72 °C (first 4 cycles); 25 s at 91 °C, 45 s at 60 °C, 30 s at 72 °C (next 26 cycles); 25 s at 91 °C, 60 s at 55 °C, 120 s at 72 °C (last 5 cycles) and finally a 10-min extension at 72 °C. The presence or absence of PCR products was determined following separation on a 2% agarose gel containing 0.5 μg/ml ethidium bromide.

2.4. HLA-Cw allele genotyping

The genotyping of HLA-C was performed using the amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) method. Each reaction contained a primer mixture consisting of allele- or group-specific primer pairs as well as four internal control primers matching nonallelic sequences (exon 5 of APC gene). Specific amplification of the HLA-C gene was performed using 23 primers for HLA-C.

PCR was carried out in a reaction volume of 13 μl containing 100 ng genomic DNA, 0.8 × buffer (40 mM KCl, 1.2 mM $MgCl_2$, 8.0 mM Tris–HCl pH 8.8, 0.08% Triton X-100), 5% dimethyl sulfoxide (DMSO), 200 μM of each dNTP, 0.25 U Taq DNA polymerase (iNtRON biotechnology, Seongnam, Korea), 1 μM of each sequence-specific primer and 0.2 μM of internal control primers. The amplifications were performed in a GeneAmp PCR system 9600 (Perkin–Elmer Corporation). A total of 30 cycles were used for the amplification with the following steps: heating to 96 °C for 1 min to denature the DNA, denaturation at 96 °C for 25 s, annealing at 70 °C for 45 s and extension at 72 °C for 30 s (for the first 5 cycles); 96 °C for 25 s, 65 °C for 45 s, 72 °C for 30 s (for the next 21 cycles); 96 °C for 25 s, 55 °C for 60 s, 72 °C for 120 s (for the last 4 cycles); and a final 1 min extension at 72 °C. The presence or absence of PCR products was determined following separation on a 1.5% agarose gel containing 0.5 μg/ml ethidium bromide.

HLA-C allele can be divided into HLA-C1 group that carries an asparagines residue at position 80 (HLA-Cw01, 03, 07, 08, 09, 10, 12, 14) and HLA-C2 group that has a lysine residue at position 80 (HLA-Cw02, 04, 05, 06, 15) [9].

2.5. Statistical analysis

When the values were greater than 5, chi-square tests were applied to compare the frequencies of the KIR genes, and two-tailed Fisher's exact tests were applied if the values were less than 5. The relative risk was calculated using Woolf and Haldane's methods, and considered significant if the *p*-value was less than 0.05.

3. Results

3.1. Clinicopathologic findings on the colorectal cancer patients

A total of 241 colorectal cancer patients were enrolled. There were 135 male patients (56%), and 106 female patients (44%). The mean age was 61.7 ± 11.1 years old. The location of the tumor was the colon in 138 patients (57.3%), and the rectum in 103 patients (42.7%). The TNM stage was 0 in 4 patients, I in 36 patients, II in 71 patients, III in 99 and IV in 31 patients. There were 226 patients with adenocarcinoma, 14 with mucinous adenocarcinoma. The tumor differentiation was well-differentiated in 17 cases, moderately-differentiated in 188 cases, poorly-differentiated in 18 cases, mucinous in 14 (Table 1).

3.2. Genetic polymorphisms of KIR genes among ethnic groups

KIR genotype of the control group of this study was compared to the control group of the Western studies. In the Korean group, the frequency of KIR2DL2 (12.6% vs. 46.7%, $p < 0.0001$) and KIR2DS2 (19.5% vs. 47.5%, $p < 0.002$) were lower, and KIR2DL3 (100% vs. 91.0%, $p < 0.0001$) was higher than in the Western group [15,16]. But the rates of the KIR genotype of this study's control group were similar to the Japanese data [22], so there were ethnic differences in the KIR genotypes (Table 2).

3.3. Genetic polymorphisms of KIR genes of colorectal cancer patients

KIR2DL1, KIR2DL4, KIR3DL2, KIR3DL3, KIR2DP1, and KIR3DP1 were always found, in both the control and the colorectal cancer patients group. Average KIR genes per patient were 10.75 ± 2.0 (range, 9–15) in the normal control group, and 10.9 ± 2.0 (range, 9–16) in the colorectal cancer patients group. The frequency of KIR2DS5 (33.2% vs. 20.8%, *p*-value < 0.007, OR = 1.9) was higher in the colorectal cancer group than in the normal control group (Table 3).

The colorectal cancer group was divided into a colon cancer group and a rectal cancer group and compared to the control group. In the colon cancer group, the frequency of KIR2DS5 (*p*-value < 0.02, OR = 1.91) was higher than in the control group. In the rectal cancer group, the frequency of KIR2DS5 (*p*-value < 0.03, OR = 1.88) was higher than in the control group, and the frequencies of KIR3DL1 (*p*-value < 0.05, OR = 0.26), KIR2DS2 (*p*-value < 0.01, OR = 0.35) and KIR2DS4 (*p*-value < 0.05, OR = 0.26) were significantly lower than in the control group. Comparing the colon and rectal cancer subgroups, the frequency of KIR2DS2 was lower in the rectal cancer group than in the colon cancer group ($p < 0.05$, Table 3).

By dividing colorectal cancer group according to the tumor differentiation, it was found that there were no difference in the frequencies of KIRs in the poorly-differentiated group, but the frequency of KIR2DS5 (*p*-value < 0.02, OR = 1.80) was higher in the well or moderately-differentiated group (Table 4). In the rectal cancer group, the frequency of KIR2DS3 (*p*-value < 0.03, OR = 0.39) was lower, and the frequency of KIR2DS5 (*p*-value < 0.03, OR = 1.97) was higher than in the control group (Table 4). Also, the frequency of KIR2DS3 was lower in rectal cancer group than colon cancer group ($p < 0.03$).

3.4. Comparison of HLA-Cw allele between the colorectal cancer patients and control group

The frequency of HLA-Cw6 (9.1% vs. 15.7%, *p*-value < 0.05, OR = 0.54) and HLA-Cw7 (17.4% vs. 27.7%, *p*-value < 0.02, OR = 0.55) were lower in the colorectal cancer group than in the normal control group. There was no difference of the frequencies of HLA-Cw in colon cancer group, but in the rectal cancer group, the frequency of HLA-Cw7 (16.5% vs. 27.7%, *p*-value < 0.04, OR = 0.52) was lower than in the control group (Table 5). There were differences between the colon cancer and rectal cancer group in HLA-Cw14 allele (*p*-value < 0.03). However, there were no differences between the colorectal cancer group and normal control group when HLA-Cws were classified into the HLA-C1 or HLA-C2 group. In addition, there were no differences in the frequencies of HLA-C1/C2 homozygous or heterozygous.

Table 1
Characteristics of the colorectal cancer patients.

Clinicopathological parameters	*N* (%)
Gender	
Male	135 (56%)
Female	106 (44%)
Tumor location	
Colon	138 (57.3%)
Rectum	103 (42.7%)
Cell type	
Adenocarcimona	226 (93.8%)
Mucinous carcinoma	14 (5.8%)
Differentiation	
Well	17
Moderately	188
Poorly	18
Mucinous	14
TNM Stage	
Tis	4 (1.7%)
I	36 (14.9%)
II	71 (29.5%)
III	99 (41.1%)
IV	31 (12.9)

Table 2
Frequencies of KIR genes of normal control group in the present study, compare to those in the Western, and Japanese studies.

Function	KIR gene		Korean *N* = 159 (%)	UK[11] *N* = 255 (%)	Japan[13] *N* = 41 (%)
Inhibitory	2DL	1	100.0	96.9	100
		2	12.6	46.7†	14.6
		3	100.0	91.0+	100
		4	100.0	100	100
		5	37.1	46.3	39.0
	3DL	1	98.1	94.9	97.6
		2	100.0	100	100
		3	100.0	100	100
Activating	2DS	1	35.2	38.0	34.1
		2	19.5	47.5‖	14.6
		3	18.2	25.1	14.6
		4	98.1	94.9	97.6
		5	20.8	30.6	24.4
	3DS	1	35.2	38.0	29.3
Pseudo	2DP	1	100.0	96.9	NR
	3DP	1	100.0	100	NR

NR = not reported, † *p*-value = 0.000, + *p*-value = 0.002, ‖ *p*-value = 0.000.

Table 3
Frequencies of KIR genes in colon and rectal cancer patients.

Function	KIR gene		Colorectal cancer $N = 241$ (%)	Colon $N = 138$ (%)	Rectum $N = 103$ (%)	Normal control $N = 159$ (%)
Inhibitory	2DL	1	241 (100.0)	138 (100.0)	103 (100.0)	159 (100.0)
		2	27 (11.2)	20 (14.5)	7 (6.8)	20 (12.6)
		3	240 (99.6)	137 (99.3)	103 (100.0)	159 (100.0)
		4	241 (100.0)	138 (100.0)	103 (100.0)	159 (100.0)
		5	107 (44.4)	61 (44.2)	46 (44.7)	59 (37.1)
	3DL	1	229 (95.0)	133 (96.4)	96 (93.2)‖	156 (98.1)
		2	241 (100.0)	138 (100.0)	103 (100.0)	159 (100.0)
		3	241 (100.0)	138 (100.0)	103 (100.0)	159 (100.0)
Activating	2DS	1	106 (44.0)	58 (42.0)	48 (46.6)	56 (35.2)
		2††	31 (12.9)	23 (16.7)	8 (7.8)¶	31 (19.5)
		3	32 (13.3)	19 (13.8)	13 (12.6)	29 (18.2)
		4	229 (95.0)	133 (96.4)	96 (93.2)**	156 (98.1)
		5	80 (33.2)†	46 (33.3)+	34 (33.0)++	33 (20.8)
	3DS	1	99 (41.1)	53 (38.4)	46 (44.7)	56 (35.2)
Pseudo	2DP	1	241 (100.0)	138 (100.0)	103 (100.0)	159 (100.0)
	3DP	1	241 (100.0)	138 (100.0)	103 (100.0)	159 (100.0)

† p-value < 0.007, Odd ratio (OR) = 1.9, + p-value < 0.02, OR = 1.91, ‖ p-value < 0.05, OR = 0.26.
¶ p-value < 0.01, OR = 0.35, ** p-value < 0.05, OR = 0.26, ++ p-value < 0.03, OR = 1.88.
††p-value<0.05 (colon vs. rectum).

Table 4
Frequencies of KIR genes in colon and rectal cancer patients according to the tumor differentiation.

Function	KIR gene		Well or moderately differentiated			Poorly differentiated			Normal
			Colorectal cancer	Colon	Rectum	Colorectal cancer	Colon	Rectum	
			$N = 203$ (%)	$N = 115$ (%)	$N = 88$ (%)	$N = 18$ (%)	$N = 14$ (%)	$N = 4$ (%)	$N = 159$ (%)
Inhibitory	2DL	1	203 (100.0)	115 (100.0)	88 (100.0)	18 (100.0)	14 (100.0)	4 (100.0)	159 (100.0)
		2	22 (10.8)	12 (10.4)	10 (11.4)	3 (16.7)	2 (14.3)	1 (25.0)	20 (12.6)
		3	202 (99.5)	114 (99.1)	88 (100.0)	18 (100.0)	14 (100.0)	4 (100.0)	159 (100.0)
		4	203 (100.0)	115 (100.0)	88 (100.0)	18 (100.0)	14 (100.0)	4 (100.0)	159 (100.0)
		5	90 (44.3)	54 (47.0)	36 (40.9)	5 (27.8)	4 (28.6)	1 (25.0)	59 (37.1)
	3DL	1	193 (95.1)	110 (95.7)	83 (94.3)	17 (94.4)	14 (100.0)	3 (75.0)	156 (98.1)
		2	203 (100.0)	115 (100.0)	88 (100.0)	18 (100.0)	14 (100.0)	4 (100.0)	159 (100.0)
		3	203 (100.0)	115 (100.0)	88 (100.0)	18 (100.0)	14 (100.0)	4 (100.0)	159 (100.0)
Activating	2DS	1	89 (43.8)	52 (45.2)	37 (42.0)	5 (27.8)	4 (28.6)	1 (25.0)	56 (35.2)
		2	25 (12.3)	14 (12.2)	11 (12.5)	3 (16.7)	2 (14.3)	1 (25.0)	31 (19.5)
		3	29 (14.3)	22 (19.1)¶	7 (8.0)+	1 (5.6)	1 (7.1)	0 (0.0)	29 (18.2)
		4	193 (95.1)	110 (95.7)	83 (94.3)	17 (94.4)	14 (100.0)	3 (75.0)	156 (98.1)
		5	65 (32.0)†	35 (30.4)	30 (34.1)‖	4 (22.2)	3 (21.4)	1 (25.0)	33 (20.8)
	3DS	1	84 (41.4)	50 (43.5)	34 (38.6)	5 (27.8)	4 (28.6)	1 (25.0)	56 (35.2)
Pseudo	2DP	1	203 (100.0)	115 (100.0)	88 (100.0)	18 (100.0)	14 (100.0)	4 (100.0)	159 (100.0)
	3DP	1	203 (100.0)	115 (100.0)	88 (100.0)	18 (100.0)	14 (100.0)	4 (100.0)	159 (100.0)

† $P < 0.02$, OR = 1.80; + $P < 0.03$, OR = 0.39; ‖ $P < 0.03$, OR = 1.97.
¶ $P < 0.03$ (colon vs. rectum).

Table 5
Frequencies of HLA-Cw type in colorectal cancer patients.

	Colorectal cancer	Colon	Rectum	Normal control
	$N = 241$ (%)	$N = 138$ (%)	$N = 103$ (%)	$N = 159$ (%)
HLA-C1 group				
HLA-Cw1	80 (33.2)	47 (34.1)	33 (32.0)	59 (37.1)
HLA-Cw7	42 (17.4)+	25 (18.1)	17 (16.5)‖	44 (27.7)
HLA-Cw8	53 (22.0)	33 (23.9)	20 (19.4)	29 (18.2)
HLA-Cw9	52 (21.6)	31 (22.5)	21 (20.4)	23 (14.5)
HLA-Cw10	69 (28.6)	37 (26.8)	31 (30.1)	39 (24.5)
HLA-Cw12	10 (4.1)	5 (3.6)	5 (4.9)	11 (6.9)
HLA-Cw14¶	58 (24.1)	26 (18.8)	32 (31.1)	34 (21.4)
HLA-C2 group				
HLA-Cw2	1 (0.4)	0 (0.0)	1 (1.0)	0 (0.0)
HLA-Cw4	32 (13.3)	21 (15.2)	11 (10.7)	21 (13.2)
HLA-Cw5	9 (3.7)	6 (4.3)	3 (2.9)	6 (3.8)
HLA-Cw6	22 (9.1)†	12 (8.7)	10 (9.7)	25 (15.7)
HLA-Cw15	19 (7.9)	11 (8.0)	8 (7.8)	8 (5.0)
HLA-C1C1	161 (66.8)	90 (65.2)	71 (68.9)	105 (66.0)
HLA-C1C2	74 (30.7)	46 (33.3)	28 (27.2)	48 (30.2)
HLA-C2C2	6 (2.5)	2 (1.4)	4 (3.9)	6 (3.8)

† p-value < 0.05, OR = 0.54, + p-value < 0.02, OR = 0.55, ‖ p-value < 0.04, OR = 0.52.
¶ p-value < 0.03 (colon vs. rectum).

3.5. The combination of KIR genotype and HLA-Cw allele

The frequency of KIR2DS2 with HLA-C1 homozygous was lower in the colorectal cancer group than in the normal control group (5.8% vs. 14.5%, p-value < 0.004, OR = 0.36, Table 6). There were no differences in the frequencies of the KIR gene in colon cancer group, but in rectal cancer group, the frequency of KIR2DS2 with HLA-C1 was decreased significantly (2.9% vs. 14.5%, p-value < 0.004, OR = 0.18, Table 6).

4. Discussion

NK cells that express CD56 in the absence of CD3 provide front-line defense against viral infections and cancer cells without previous sensitization [3,4].

There are many receptors on the surface of human NK cells. Of these, KIR, which recognizes HLA class I as their ligands, can activate or inhibit NK cells plays an important role in controlling the function of NK cells [5].

It is known that there are ethnic differences in the KIR genotype. In the present study, we compare the frequencies of KIR gene of the

Table 6
The association of KIR genes and their ligands between colorectal cancer patients and control.

	Colorectal cancer	Colon	Rectum	Normal control
	$N = 241$ (%)	$N = 138$ (%)	$N = 103$ (%)	$N = 159$ (%)
KIR2DL1 with HLA-C2	80 (33.2)	48 (34.8)	32 (31.1)	54 (34.0)
with HLA-C2C2	6 (2.5)	2 (1.4)	4 (3.9)	6 (3.8)
KIR2DL2 with HLA-C1	26 (10.8)	20 (14.5)	6 (5.8)	19 (12.0)
with HLA-C1C1	12 (5.0)	10 (7.2)	2 (1.9)	12 (7.5)
KIR2DL3 with HLA-C1	234 (97.1)	135 (97.8)	99 (96.1)	153 (96.2)
with HLA-C1C1	161 (66.8)	90 (65.2)	71 (68.9)	105 (66.0)
KIR2DS1 with HLA-C2	34 (14.1)	24 (17.4)	10 (9.7)	22 (13.8)
With HLA-C2C2	1 (0.4)	1 (0.7)	0 (0.0)	3 (1.9)
KIR2DS2 with HLA-C1	30 (12.4)	23 (16.7)	7 (6.8)+	30 (18.9)
With HLA-C1C1	14 (5.8)†	11 (8.0)	3 (2.9)‖	23 (14.5)

† p-value < 0.004, OR = 0.36, + p-value < 0.007, OR = 0.31, ‖ p-value < 0.004, OR = 0.18.

Korean normal control group with those of the English and Spanish studies [15,16]. In the Korean populations, the frequencies of KIR2DL2 and KIR2DS2 were lower than in the Western groups, and the frequency of KIR2DS3 was higher. But there was no difference in the frequency of KIR genes between the Korean and Japanese control groups [22]. So we could confirm the presence of ethnic differences in KIR genes (Table 2).

In the present study, we tested eight inhibiting KIR genes (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3), six activating KIR genes (2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1), and two pseudo-genes (2DP1, 3DP2). We found that the frequency of the KIR2DS5 gene was higher in colorectal cancer group, this difference was observed in the well or moderately-differentiated tumor subgroup. In the rectal cancer subgroup, the frequency of KIR2DS5 was higher, and the frequencies of KIR3DL1, KIR2DS2, and KIR2DS4 were lower than normal control group. For this reason, there may be some different immune mechanisms in cancer developments between colon and rectal cancer.

Similar to our study's results, Paladino et al. reported that the frequency of KIR2DS5 was higher, and the frequencies of KIR2DL2 and KIR2DS2 were lower in hepatitis C patients [23]. But Nowak et al. reported that the frequency of KIR2DS5 was lower when there was ankylosing spondylitis, endometriosis, and renal transplant rejection [24]. So we can deduce that KIR2DS5 may play a different role in different diseases.

KIRs bind to specific HLA-C as their ligand. KIR2DL1 and KIR2DS1 are known to bind to HLA-C2, and KIR2DL3, KIR2DS2, and KIR2DL2 are known to bind to HLA-C1 [10]. But there are differences in the inhibiting effect among KIRs. KIR2DL1/HLA-C2 binding is the most powerful, followed by KIR2DL2/HLA-C1, KIR2DL3/HLA-C1 [10].

In the present study, we compared the frequency of KIR according to KIR genotype and its ligand HLA-Cw combination. The frequency of KIR2DS2 was lower in the HLA-C1 homozygous group (5.8% vs 14.5%, p-value < 0.004, OR = 0.36), and in the rectal cancer subgroup, the frequency of the KIR2DS2/HLA-C1 combination was lower (Table 6). Similarly to our findings, Middleton et al. reported that KIR2DS2 or KIR2DL2 was lower with HLA-C1 group in leukemic patients [25]. They also concluded that the HLA-C1/KIR2DS2 combination can reduce the risk of leukemia. Accordingly, we can deduce that the HLA-C1 and KIR2DS2 combination can activate NK cells and plays some role in protecting against the development of colorectal cancer.

In the present study, the frequencies of HLA-Cw6 and HLA-Cw7 were lower in colorectal cancer group than in the normal control group. But after classification into an HLA-C1 and an HLA-C2 group, there was no difference between the colorectal cancer and normal control group.

There are several shortcomings to our study. The first is the limitation of the genetic study; specifically, we cannot know how exactly KIR genes are expressed on the cell surface. For this reason, further studies are needed to investigate the expression of KIR on NK cells. The other drawback is the possibility of other ligands to KIRs. Currently, not all of the ligands to KIRs are elucidated. Katz et al. reported that in malignant melanoma patients, there is another ligand to KIR2DS4 other than HLA [26]. In the present study, the frequency of KIR2DS5 was higher in colorectal cancer patients. However its ligand is not known yet, therefore further studies are needed to find the ligand of KIR2DS5.

In conclusion, the frequency of KIR2DS5 is higher in Korean colorectal cancer patients, and in the rectal cancer subgroup, the frequencies of KIR3DL1, KIR2DS2 and KIR2DS4 are lower, there may be some immunologic differences between colon and rectal cancers. And among the patients with HLA-C1 homozygote, the frequency of KIR2DS2 is decreased. Therefore, KIR2DS2 in presence of its ligand (HLA-C1 group) may have a protective effect against colorectal cancer.

What is already known on this topic?

KIR2DS5 is higher in Korean colorectal cancer patients. And KIR2DS2 in presence of its ligand may have a protective effect against colorectal cancer.

Ethical approval

The Institutional Review Board of the Catholic University of Korea (KC12TISI0302).

Conflicts of interest

None.

Funding

A grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare &Family Affairs, Republic of Korea (A092258) 2011 research grant from St. Vincent's Hospital Research Institute Of Medical Science.

Author contribution

Hyung-Jin Kim: data collection & analysis, writing
Hae-Baeg Choi, Jung-Pil Jang, In-Cheol Beak: data collection, data analysis
Eun-Jeong Choi: study design
Miyoung Park: study design
Tae-Gyu Kim: study design, data analysis
Seong-Taek Oh: study design, data analysis

Acknowledgment

This study was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare &Family Affairs, Republic of Korea (A092258), and by a 2011 research grant from St. Vincent's Hospital Research Institute of Medical Science.

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