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Activating and inhibitory killer cell immunoglobulin like receptors *(KIR)* genes are involved in an increased susceptibility to colorectal adenocarcinoma and protection against invasion and metastasis

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ABSTRACT

Background: : A set of activating and inhibitory *KIRs* (*aKIR, iKIR*) are involved in NK cell mediated immunity. This study was carried out in order to investigate the KIRs pattern and its association with colorectal carcinoma (CRC) development and clinical outcomes.
Methods: : Sequence-specific primers-polymerase chain reaction (SSP-PCR) for typing of 16 *KIR* genes was utilized in 165 patients with colorectal adenocarcinoma with 165 age and gender matched healthy controls (CNs).
Results: : Possessing *KIR2DS1, 2DS5, 3DS1, 2DS4fl, 2DL5*, telomeric half *KIR* genes, ≥ 4 *aKIR* and CXT4 genotype were associated with an increased susceptibility to colorectal adenocarcinoma while *KIR2DS4del* and *iKIR* > *aKIR* confer resistance to CRC. On the other hand, clinical associations revealed the defensive role of telomeric *KIR3DL1, 3DS1, 2DS1, 2DS4,* genotypes with ≥ 4 *aKIR* and more inhibitory *KIRs* than activating ones (I > A) against metastasis and CXTX genotype in perineural invasion.
Conclusion: : According to current results it appears that KIRs system play distinctive roles in development and metastasis of colorectal adenocarcinoma.

1. Introduction

Colorectal cancer (CRC) is characterized by development of malignant epithelial tumors in colon and rectum, with adenocarcinoma being most common (95%) ; it accounts for the second most common cause of cancer in females and the third in males across the world (Bhandari et al., 2017).

Both genetic and environmental factors play a major role in the aetiology of CRC. Presumably, disparate environmental factors that influence colorectal carcinogenesis, describes the tremendous heterogeneity of tumor (Yamagishi et al., 2016). From a genetic viewpoint, CRC is a complex disease, and molecular and genetic alterations are often result in development of invasive adenocarcinoma (adenoma) from premalignant lesion (Vogelstein et al., 1988). Which usually takes between 10 to 15 years in different backgrounds such as individual's immune system, gut microbiota (tumor microenvironment) as well as chronic inflammatory state in surrounding histologically normal tissues which might have a significant impact on tumor development and progression (Jones et al., 2008; Kuipers et al., 2015; Lasry et al., 2016).

Despite applying colonoscopy as a gold standard for diagnosis of colorectal cancer which has a high diagnostic accuracy and is also the only screening method that provides therapeutic effect (Morris et al., 2015; Pox et al., 2012) but the mortality rates are still high globally, mainly due to recurrence or distant metastasis (Yan et al., 2018). Hence, it is urgent need to stimulate research for discovering molecular data in CRC pathogenesis, which emphasis on the association between genetic factor and tumor development and progression. (Ogino et al., 2011). Several immune regulating mediators secreted from various cells within tumor microenvironment contributes to altering immune functions (Accomando et al., 2012; Levy et al., 2011).

NK cells are cytotoxic lymphocytes which kill target cells with reduced MHC class I molecules expression as a mechanism of protection against malignant transformation (Levy et al., 2011). The existence of activating and inhibitory Killer cell immunoglobulin-like receptors (KIR) which recognize normally expressed HLA molecules control the activation of NK cells (Moretta and Moretta, 2004). The KIR gene family is found on chromosome 19 and encodes receptors with either two (2D) or three (3D) extracellular immunoglobulin-like domains.

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Inhibitory receptors possess a long (L) cytoplasmic tail while activating receptors having a short (S) cytoplasmic tail (Boyton and Altmann, 2007; Martinez-Borra and Khakoo, 2008).

Individuals inherit different numbers and types of *KIR* genes which recognize polymorphic HLA epitopes (Moretta and Moretta, 2004). A variety of forces in encounter with pathogens are major driving factors for evolution of diverse and rapidly evolving KIR receptors (Khakoo et al., 2000). Fourteen *KIR* genes encoding receptors with either inhibitory (3DL1-3, 2DL1-3, 2DL5) or activating functions (3DS1, 2DS1-5), or both inhibitory as well as activating properties (2DL4), and two non-functional pseudogenes (2DP1, 3DP1) have been identified. Inhibitory KIR receptors recognize HLA class I molecules with distinct specificities and trigger effector signaling to block NK cell activity and the ligands for activating KIRs are a matter of study yet (Rajalingam, 2011).

A number of studies have investigated the associations between inhibitory and activating *KIR* as well as their HLA ligands with cancer incidence and prognosis both in patients with solid tumors and hematologic malignancies (Mehta et al., 2018; Morales-Estevez et al., 2016; Babor et al., 2013). In consideration of the above, KIR/HLA relationships that mostly generate net inhibitory signals are over represented in patients with acute and chronic leukemia, Hodgkin lymphoma, melanoma and breast cancer, and this might be reflected in matched autologous inhibitory KIR/HLA interactions which avoid lysis of malignant cells in some extent; in contrast to the expression of activating *KIRs* in some conditions which is associated with superior outcomes in various malignancies (Marcenaro et al., 2013, Ozturk et al., 2012).

Prior studies have proposed that activating *KIR* genes (Canossi et al., 2016) and Bx KIR haplotypes confer reduced risk for CRC, certain activating *KIRs* (2DS2 and 2DS3) in the presence of their ligands suggest a genotype that provided protection against tumor recurrence (Beksac et al., 2015). However, these studies are limited by small populations or multi ethnic cohorts. Hence, in order to achieve statistical conclusion validity on *KIRs* role in CRC development, we analyzed a larger population as well as ethnically homogeneous case and controls (CNs) which can prevent high rates of false positives due to population stratification.

2. Materials and methods

2.1. Study subjects, DNA extraction and KIR genotyping

A case-control study was performed on 330 eligible subjects in Fars province of Iran.

One hundred and sixty five histologically and pathologically confirmed cases of colorectal adenocarcinoma were recruited from Faghihi Hospital affiliated with Shiraz University of Medical Sciences, along with 165 age and sex matching healthy controls (CNs) from a highly homogeneous population of Iran (Fars) as comparison group to avoid population stratification issue leading to false positive results. The individuals who had inflammatory bowel disease including ulcerative colitis, crohn's disease and coexistence of another type of tumor/autoimmune diseases were excluded at the beginning of study design. We selected healthy controls without familial history of colorectal cancer.

Genomic DNA was extracted from whole blood and KIR genotyping was performed by SSP-PCR method using specific KIR primers designed by Vilches et al. (Vilches et al., 2007) and Ashouri et al. (Ashouri et al., 2009) according to our previous data (Barani et al., 2018).

Histological invasion pattern particularly lymphatic, vascular and perineural invasion and tumor metastasis were assessed in affected patients as probable prognostic factor for colorectal adenocarcinoma. The demographic and clinical characteristics of the study population were gathered from datasheets; informed consent was obtained from research participants prior to initiating any activities; and ethical approval of the research was confirmed by Medical Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1397.424).

2.2. Statistical analysis

Data analysis was performed by using the SPSS version 16.0 software. The frequency of each gene and haplotypes was compared between the colorectal adenocarcinoma patients and CNs by Yates's chi-squared test in order to minimize the differences. The $p < 0.05$ was considered significant for two-tailed Chi square with Yates' correction. Odds ratio (OR) and 95% confidence interval (CI) were estimated in each statistical test.

3. Results

3.1. Demographic characteristics

The mean age for patients with colorectal adenocarcinoma was 54.58 ± 14.68 and 55.69 ± 13.98 for healthy controls. Females made up 35.5% of patients and 65.5% were males. Considering the epidemiological findings that cancer developing increases with age, we categorized colorectal adenocarcinoma patients into two distinct age groups: young and older adults, individuals with ≤45 years old (26.1%) and those with over 45 year's old (73.9%) respectively.

3.2. Association of KIR2DS1, 2DS5, 3DS1, 2DS4fl and 2DL5 with an increased susceptibility to colorectal adenocarcinoma and the protecting role of KIR2DS4del

Distribution of A and B haplotypes associated KIR genes were compared between 165 patients with colorectal adenocarcinoma and 165 healthy controls (Table 1). Furthermore, distribution of *KIR2DS4* and variants were evaluated by categorizing the individuals into 3 groups: **A)** *2DS4fl*: individuals possessing just full-length *KIR2DS4*, **B)** *2DS4del*: individuals possessing just deleted variant of *KIR2DS4* and **C)** *2DS4 fl, del*: individuals possessing both full-length and deleted variant of *KIR2DS4* simultaneously.

A significant increase was observed in the carrier frequency of *KIR2DS1* ($p = 0.0011$, OR = 2.16, CI = 1.38–3.98), *2DS5* ($p = 0.0265$, OR = 1.73, CI = 1.09–2.75), *3DS1* ($p = 0.0010$, OR = 2.18, CI = 1.39–3.44) and *2DL5* ($p = 0.0328$, OR = 1.66, CI = 1.06–2.59) from B haplotype and *2DS4fl* ($p = 0.0001$, OR = 5.14, CI = 3.22–8.22) of A haplotype in patients with colorectal adenocarcinoma compared to healthy controls which revealed their positive association with CRC (Table 1) while the decreased frequency of *KIR2DS4del* in CRC patients compared to healthy controls (38.20% vs. 87.90%, $p = 0.0001$, OR = 0.85, CI = 0.05-0.15), made it known as protective gene which might play a role in CRC resistance.

3.3. Telomeric half KIR genes and CXT4 genotype confer susceptibility to CRC

According to the presence/absence of activating and inhibitory *KIRs* gene, the AA and Bx haplogroups were defined in study population and on the basis of C4 and T4 clusters, the Bx genotypes were subdivided into 4 subsets. There was no remarkable difference in the frequencies of AA and Bx haplogroups. Regarding to Bx genotypes, CXT4 genotype had a higher frequency in CRC than CNs ($p = 0.002$, OR = 2.9, CI = 1.49–5.63). Consequently, the carrier frequency of telomeric half KIR genes was enhanced in colorectal adenocarcinoma compared to healthy controls ($p = 0.0163$, OR = 1.91, CI = 1.15–3.17). Obviously, the higher frequency of CXT4 genotype (Table 3) in patients was driven by a decline in telomeric half *KIR* genes (Table 2). No significant difference was observed in the frequencies of A, B haplogroups and C4, T4 gene clusters between cases and CNs (Table 3). Ultimately, 55 distinct KIR genotypes including 33 common and 22 unique ones in 330 individuals (**Supplementary** Tables 1 and 2) were characterized by referring to the allele frequency database (http://www.allelefrequencies.net).

Table 1
Comparing the carrier frequency of *KIR* genes in CRC and CN.

KIRs gene		CRC n = 165 N %F	CN n = 165 N %F	CRC vs. CN P-value OR CI
A haplotype associated genes	***2DL1***	163 98.80%	165 100%	NS
	2DL3	145 87.90%	146 88.50%	NS
	3DL1	152 92.10%	159 96.40%	NS
	2DS4	156 94.50%	159 96.40%	NS
	2DS4 fl	113 68.50%	49 29.70%	0.0001* 5.14 3.22-8.22
	2DS4 del	63 38.20%	145 87.90%	0.0001* 0.85 0.05-0.15
	2DS4 fl, del	21 12.70%	34 20.6%	NS
B haplotype associated genes	***2DS1***	80 48.50%	50 30.30%	0.0011* 2.16 1.38-3.98
	2DS2	99 60.0%	99 60.0%	NS
	2DS3	79 47.90%	68 41.20%	NS
	2DS5	65 39.4%	45 27.3%	0.0265* 1.73 1.09-2.75
	3DS1	78 47.30%	48 29.10%	0.0010* 2.18 1.39-3.44
	2DL2	105 63.60%	102 61.8%	NS
	2DL5	109 66.1%	89 53.90%	0.0328* 1.66 1.06-2.59

2DS4del: *KIR2DS4* deleted variant, *2DS4fl: KIR2DS4* full length, *2DS4fl, del*: both *KIR2DS4* deleted variant and full length.

* P < 0.05: statistically significant; based on two-tailed Chi-square with Yates' correction; NS = non-significant.

Table 2
Comparison of different combinations of *KIRs* between CRC and CNs.

KIR genes number		CRC n = 165	CN n = 165	CRC vs. CN
Different number and combination of KIRs gene		N %F	N %F	P-value OR CI
iKIR > aKIR		93 56.4%	119 72.1%	0.0041* 0.5 0.31 to 0.79
aKIR > iKIR		35 21.2%	26 15.8%	NS
≥ 4 a KIR		82 49.7%	53 32.1%	0.0017* 2.09 1.33 to 3.26
Centromeric half *KIR* genes (*KIR2DL1, 2DL2, 2DL3, 2DS2*)	Neg	87 52.70%	85 51.50%	NS
	Pos	78 47.30%	80 48.50%	
Telomeric half *KIR* genes (*KIR3DL1, 3DS1, 2DS1, 2DS4*)	Neg	113 68.50%	133 80.60%	0.0163* 1.91
	Pos	52 31.50%	32 19.40%	1.15 to 3.17

* P < 0.05: statistically significant; based on two-tailed Chi-square with Yates' correction.

3.4. Strong positive association of ≥ 4 aKIR and negative association of iKIR > aKIR with CRC risk

According to inherited KIR haplotypes, individuals have distinct gene content with different numbers of activating and inhibitory *KIRs* gene. In order to explore whether any significant difference is present in the number of activating and inhibitory KIR genes (*aKIRs*, *iKIRs*) between case and controls, we assessed various comparisons with different number of activating and inhibitory KIRs. As shown in Table 2, individuals with higher number of *iKIRs* than *aKIRs* constituted a much lower proportion of patients with CRC in comparison to healthy controls (p = 0.0041, OR = 0.5, CI = 0.31 to 0.79) while a highly significant increase was observed in CRC patients having ≥4 *aKIR* compared to CNs (p = 0.0017, OR = 2.09, CI = 1.33–3.26). It was disclosed that, there are negative association of *iKIR* > *aKIR* whereas a positive association of ≥4 *aKIR* with CRC risk.

Table 3
The frequency of the KIR genotypes and haplotypes in the study population.

KIR genotypes, haplogroups and clusters	CRC n = 165 N %F	CN n = 165 N %F	CRC vs. CN P-value OR CI
AA haplogroup	29 17.6%	43 26.10%	NS
Bx haplogroup	136 82.40%	122 73.90%	
C4Tx genotype	38 23.0%	41 24.80%	NS
CxT4 genotype	35 21.20%	14 8.50%	0.0020* 2. 9 1.49-5.63
C4T4 genotype	21 12.70%	20 12.10%	NS
CxTx genotype	42 25.50%	47 28.50%	NS
A haplotype	163 49.39%	183 55.45%	NS
B haplotype	167 50.61%	147 44.54%	NS
C4 gene cluster	59 35.7%	61 36.9%	NS
T4 gene cluster	56 33.9%	34 20.6%	NS

The frequency of the haplotype A & B were determined by the following formulas:
Haplotype A: 2nAA + nAB/2 N, Haplotype B: 2nBB + nAB/2 N.
(nAA: number of AA genotype, nAB: number of AB genotype, nBB: number of BB genotype).
C4 gene cluster = nC4Tx + nC4T4, T4 gene cluster = nCxT4 + nC4T4.
(n: number of people who have that subtype in each groups).
* P < 0.05: statistically significant; based on two-tailed Chi square with Yates' correction; NS = non-significant.

3.5. Demographic characteristics alter the association levels between KIR system and CRC

Considering the potential effects of epidemiological factors on cancer outcomes, we categorized both colorectal adenocarcinoma patients and healthy controls into 4 subgroups based on gender and age including young adults with ≤ 45 years old and older adults with over 45 year's old.

Although age-sex matching was carried out in our study, we were inclined towards the idea that controlling for the matching factors may be needed for yielding a much precise results in the analysis. The age and gender-stratified analysis (**Supplementary** Table 3) illustrated some important points. Firstly, it changed the strength of association between the certain risk factors and the outcome among different strata; regarding to the 2DS4 fl predisposing role, the odd ratio (OR) increased in young adults (6.75) and males (6.17) in comparison with total population's OR (5.14), whiles in the case of *2DS1*, *2DS5* and *3DS1*, the OR became greater in females compared to total population (*2DS1* = 3.01 to 2.16, *2DS5* = 2.7 to 1.73 and *3DS1* = 3.28 to 2.18). Secondly it removed the observed association in total population or created an association even if there were none before stratification (negative association of AA in males and CXTX in females along with

Table 4
KIR system association with colorectal adenocarcinoma invasion and metastasis.

KIRs gene and genotypes	Lymphatic invasion	Vascular invasion	Perineural invasion		Metastasis	
	n = 32 F% = 19.4%	n = 16 F% = 9.7%	n = 25 F% = 15.2%		n = 23 F% = 13.9%	
	P-value OR CI	P-value OR CI	Pos Neg	P-value OR CI	Pos Neg	P-value OR CI
I > A	NS	NS	NS		19.4% 80.6%	0.0398* 3.22 1.13 to 9.13
≥ 4 *a KIR*	NS	NS	NS		7.3% 92.7%	0.0267* 0.3 0.11 to 0.82
Telomeric *KIR* genes (*KIR3DL1, 3DS1, 2DS1, 2DS4*)	NS	NS	NS		3.8% 96.2%	0.0216* 0.17 0.04 to 0.77
CXTX	NS	NS	31% 69%	0.0022 4.15 1.71 to 10.04	NS	

* P < 0.05: statistically significant; based on two-tailed Chi square with Yates' correction; NS = non-significant.

negative association of possessing ≥ 4 *iKIR* in males with colorectal adenocarcinoma); and finally, it removed the observed association (≥4 *iKIR*) in total population.

3.6. Protecting role of certain KIRs genotypes against metastasis and perineural invasion

Histopathological features such as lymphatic, vascular and perineural invasion have been assessed as prognostic factor in colorectal adenocarcinoma patients. Overall, 19.4% of patients had lymphatic, 9.7% vascular and 15.2% perineural invasion. Besides, we evaluated metastasis as a significant prognostic factor in colorectal adenocarcinoma cases; 13.9% of them have been recorded to have distant metastasis at diagnosis.

To identify the clinical impact of *KIR* genes and genotypes in patients with colorectal adenocarcinoma, we determined the association of *KIR* genes with clinical features of CRC patients; we realized that the lower frequency of metastasis was occurred in carriers who had more inhibitory *KIRs* than activating ones (I > A), in individuals possessing four or more activating *KIRs* (≥ 4 a *KIR*) and those with telomeric *KIR* genes (*KIR3DL1, 3DS1, 2DS1, 2DS4)* **(**Table 4**)** which revealed their protective role against metastasis in patients with colorectal adenocarcinoma. Furthermore, a significant drop in perineural invasion was observed in carriers of CXTX genotype than those lack this genotype; hence, it seems that absence of CXTX genotype could intensify perineural invasion in colorectal adenocarcinoma (Table 4). No significant association was observed between lymphatic/vascular invasion and KIR system.

4. Discussion

The prevalence and mortality rates of CRC differ across the world but in general it is predicted to have an upward trend by 60%, means that the prevalence rate will reach to above 2.2 million and the mortality rate of 1.1 million by the end of next decade (Arnold et al., 2017; Barrow and Colonna, 2019). Previous researches have discussed the significant role of NK cells in both development and progression of tumors through involving in defense, homeostasis and immunosurveillance which are three urgent mechanisms in tumor immunity (Tian, 2013; Cantoni et al., 2016).

The primary findings of current research ascertained that certain *KIR* genes have an exert influence on genetic predisposition or protection against colorectal adenocarcinoma. Conforming to our results and regarding the gastrointestinal diseases, Al Omar et al., 2015 reported a higher proportion of activating *KIR2DS1*, *2DS2*, *2DS3*, *2DS5* and *3DS1* was found in CRC patients in comparison with controls (Al Omar et al., 2015). Furthermore, a study in Mexico-city found an increase frequency of *2DS1*, *2DS3*, *2DS5*, *3DS1* and *2DL5* in gastric cancer compared with asymptomatic population (Hernandez et al., 2018). Another evidence for predisposing role of *KIR2DS5* in colorectal cancer was study conducted in Korean colorectal cancer patients (Kim et al., 2014). Contrary to above mentioned studies, there was no association of KIRs with CRC in Europeans (Middleton et al., 2007) as well as Crohn's disease in Japanese population while a lower frequency of *KIR2DS4* was observed in Japanese patients with ulcerative colitis (Saito et al., 2018). The deleterious effects of certain activating *KIRs* (*KIR2DS1*, *KIR2DS2*, *KIR2DS5, KIR3DS1* and *KIR2DS4* variants) have been displayed in development of solid tumors like bladder cancer (Jamali et al., 2018), Kaposi's sarcoma (Guerini et al., 2012) breast cancer (Ozturk et al., 2012) and, our published study on head and neck squamous cell carcinoma (HNSCC) (Barani et al., 2018).

Moreover, a large body of evidence, pointing to the distinct activating *KIRs* role in pathogenesis of autoimmune diseases (Popko and GórsKa, 2015); *KIR2S2*, *KIR3DS1* and *KIR2DS1-HLA-Cw6* increased susceptibility to systemic lupus erythematous, multiple sclerosis and psoriasis respectively (Rajalingam, 2018). On the other hand, the role of activating *KIRs* in protecting against hematological malignancies have been detected which stated the presence of *KIR2DS1, 2DS3, 2DS4* and *2DS5* conferred protection in leukemia (Almalte et al., 2011; Marcenaro et al., 2013).

In respect of inhibitory KIRs, we obtained a remarkable association between *KIR2DL5* and patients with colorectal adenocarcinoma, indicate that it is also conferred in susceptibility to cancer along with activating *KIRs.* There are many reports compatible with the detrimental role of inhibitory *KIRs* particularly *KIR2DL5*, melanoma (Jiao et al., 2010), gastric cancer (Hernandez et al., 2018), ankylosing spondylitis (Jiao et al., 2010)and latent tuberculosis (Braun et al., 2015) and again in our recent study on head and neck squamous cell carcinoma (HNSCC) (Barani et al., 2018) which are in stark contrast with its protective role in hematopoietic disorders (Sugioka et al., 2016).

According to *KIR2DS4* variants, we found a strong negative association of *KIR2DS4 fl* with risk of colorectal adenocarcinoma which is accordance with HIV/AIDS researches findings (Yu et al., 2017; Olvera et al., 2015) while on the contrary to its role in graft versus host disease (GVHD) incidence after hematopoietic stem cell transplantation in patients with leukemia. Nevertheless, a greater frequency of *KIR2DS4 del* was observed in controls than cases; it means that *KIR2DS4 del* play a highly protective role against CRC which is in agreement with our previous study on HNSCC.

Frequency analysis of several comparisons with different number of activating and inhibitory *KIRs* revealed that possessing *iKIR* > *aKIR*

genotype is a potential risk factor for CRC while genotypes with ≥4 *aKIR* is associated with protection against it. Similarly, there are many reports of different *KIR* presence which demonstrated the detrimental role of ≥5 *aKIR* genes in EBV infected nasopharyngeal patients (Kovacic et al., 2005) and the beneficial potential of higher numbers of *aKIR* in children with ALL (Almalte et al., 2011).

In clinical aspects, while there was lower risk of metastasis in individuals having ≥ 4 *aKIR* and those with telomeric KIRs (*KIR3DL1, 3DS1, 2DS1, 2DS4),* the carriers of *iKIR* > *aKIR* genotype were more resistance to metastasis which is somehow controversial result and it might be explained by better performance of NK cells equipped with more inhibitory and activating receptors against CRC metastasis.

However the typical ways of tumor metastasis are vascular and lymphatic invasion, there is another route which is neoplastic invasion through and around nerves known as perineural invasion; a strong prognostic factor for CRC turned out the point that it is associated with absence of CXTX genotype which includes 10 to 13 *KIRs* in our patients with colorectal adenocarcinoma. Some studies reported the predictive role of perineural invasion in decreasing high rate of recurrence and 5-year overall survival rate in CRC (Betge and Langner, 2011). *KIR2DL5A*, *2DS5*, *2DS1*, *3DS1* had a trend towards complete response to chemotherapy as well as improved overall survival in metastatic CRC patients (De Re et al., 2014).

A lot of direct evidences for detrimental and beneficial roles of activating and inhibitory KIRs have been declared in a broad variety of different diseases ranging from cancers to autoimmune diseases which indicate that the solid tumors and autoimmune diseases possess similar *KIRs* pattern and presumably identical function due to the inflammatory context of these diseases which is in contrast with hematological malignancies. Autoimmunity and solid tumors both originate from genetic susceptibility integrated with environmental triggers, which followed by amplifying and perpetuating inflammation as the main feature of innate immunity (Rosenblum et al., 2015). It has been proved that inflammatory cytokines are predominantly participate in tumorigenesis and its progression in colorectal carcinoma as well as tumors of head and neck, skin, breast, liver and stomach (Munn, 2017).

It needs to be kept in mind that the main determinant factor for effector function of *KIRs* is interaction with specific ligands including HLA-I molecules, nonclassical MHC-I and also

non MHC-I molecules which leads to different activating and inhibitory functions of NK cells.

A possible mechanism for activating *KIRs* participation in CRC development could be tied to NK cells licensing through KIR/HLA interactions; it means that the expression of activating KIRs along with self-HLA class I molecules results in hyporesponsive state and development of anergic NK cells which are not capable in protection against tumor (disarming model), while this may be true, it is also possible that NK cells express this certain activating receptors where the specific ligands are not present, hence they are unable in recognizing and elimination of tumor cells.

Admittedly, KIR2DL5/HLA-I interactions as a potential mechanism could act in evasion and progression of colorectal adenocarcinoma cells due to fail to retain antibody-dependent cell-mediated cytotoxicity (ADCC) of NK cells.

KIR2DS4 del as a truncated soluble protein which differs from the typical KIR2DS4 by 22bp deletion, may be capable in masking the ligands for KIR2DS4 or even other regulatory cells receptors which leads to resisting to transformation and keeping down the malignant effects of KIR2DS4 fl by its modulatory role which was found in our CRC group.

Here are two different views on the role of *KIRs* in the context of chronic inflammation in CRC and other pathogen-driven cancers. As the first consideration, the dominance of inhibitory *KIRs* number (iKIRs > aKIRs) may be connected with increased susceptibility to CRC development/ progression particularly because NK cells are unable to induce a potent cytolytic activity during infection by intestinal bacteria. On the second place, the role of nonspecific inflammatory responses derived by NK cells expressing activating KIRs may be a potential mechanism predisposing to developing CRC.

The conclusion drawn from genotype association and clinical features *KIRs* is that it is plausible that increased accumulation of *aKIRs* gene (≥ 4 *aKIR*) in NK cells along with telomeric half *KIRs*: *KIR3DL1, 3DS1, 2DS1, 2DS4*) has a detrimental effect on CRC development whereas playing a favorable role in protection against metastasis and perineural invasion in patients with colorectal adenocarcinoma.

By and large, lack of enough knowledge about KIRs expression and function particularly due to unexplored activating *KIRs* ligands, making it difficult to draw firm conclusion for KIRs role in CRC development and progression. Further research on the structure and function of *KIRs* is required to pursue investigations of their roles in different stages of cancers and their application in therapeutic strategies.

Declaration of Competing Interest

The authors state no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imbio.2019.06.002.

References

Accomando, W.P., Wiencke, J.K., Houseman, E.A., Butler, R.A., Zheng, S., Nelson, H.H., Kelsey, K.T., 2012. Decreased NK cells in patients with head and neck cancer determined in archival DNA. Clinical Cancer Research, clincanres 1008.

Almalte, Z., Samarani, S., Iannello, A., Debbeche, O., Duval, M., Infante-Rivard, C., Amre, D.K., Sinnett, D., Ahmad, A., 2011. Novel associations between activating killer-cell immunoglobulin-like receptor genes and childhood leukemia. Blood 118, 1323.

Arnold, M., Sierra, M.S., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2017. Global patterns and trends in colorectal cancer incidence and mortality. Gut 66, 683.

Ashouri, E., Ghaderi, A., Reed, E., Rajalingam, R., 2009. A novel duplex SSP–PCR typing method for KIR gene profiling. Tissue Antigens 74, 62.

Babor, F., Fischer, J.C., Uhrberg, M., 2013. The role of KIR genes and ligands in leukemia surveillance. Front. Immunol. 4, 27.

Barani, S., Khademi, B., Ashouri, E., Ghaderi, A., 2018. KIR2DS1, 2DS5, 3DS1 and KIR2DL5 are associated with the risk of head and neck squamous cell carcinoma in Iranians. Hum. Immunol. 79, 218.

Barrow, A.D., Colonna, M., 2019. Exploiting NK cell surveillance pathways for cancer therapy. Cancers 11, 55.

Beksac, K., Beksac, M., Dalva, K., Karaagaoglu, E., Tirnaksiz, M.B., 2015. Impact of "killer immunoglobulin-like receptor/ligand" genotypes on outcome following surgery among patients with colorectal cancer: activating KIRs are associated with long-term disease free survival. PLoS One 10, e0132526.

Betge, J., Langner, C., 2011. Vascular invasion, perineural invasion, and tumour budding: predictors of outcome in colorectal cancer. Acta Gastroenterol. Belg. 74, 516.

Bhandari, A., Woodhouse, M., Gupta, S., 2017. Colorectal cancer is a leading cause of cancer incidence and mortality among adults younger than 50 years in the USA: a SEER-based analysis with comparison to other young-onset cancers. J. Investig. Med. 65, 311.

Boyton, R., Altmann, D., 2007. Natural killer cells, killer immunoglobulin-like receptors and human leucocyte antigen class I in disease. Clin. Exp. Immunol. 149, 1.

Braun, K., Wolfe, J., Kiazyk, S., Sharma, M.K., 2015. Evaluation of host genetics on outcome of tuberculosis infection due to differences in killer immunoglobulin-like receptor gene frequencies and haplotypes. BMC Genet. 16, 63.

Canossi, A., Aureli, A., Beato, T., Rossi, P., Franceschilli, L., Sanctis, F., Sileri, P., Lorenzo, N., Buonomo, O., Lauro, D., 2016. Role of KIR and CD16A genotypes in colorectal carcinoma genetic risk and clinical stage. J. Transl. Med. 14, 239.

Cantoni, C., Huergo-Zapico, L., Parodi, M., Pedrazzi, M., Mingari, M.C., Moretta, A.,

Sparatore, B., Gonzalez, S., Olive, D., Bottino, C., 2016. NK cells, tumor cell transition, and tumor progression in solid malignancies: new hints for NK-based immunotherapy? J. Immunol. Res. 2016.
De Re, V., Caggiari, L., De Zorzi, M., Talamini, R., Racanelli, V., D'Andrea, M., Buonadonna, A., Zagonel, V., Cecchin, E., Innocenti, F., 2014. Genetic diversity of the KIR/HLA system and outcome of patients with metastatic colorectal cancer treated with chemotherapy. PLoS One 9, e84940.
Guerini, F.R., Mancuso, R., Agostini, S., Agliardi, C., Zanzottera, M., Hernis, A., Tourlaki, A., Calvo, M.G., Bellinvia, M., Brambilla, L., 2012. Activating KIR/HLA complexes in classic Kaposi's sarcoma. Infect. Agents Cancer 7, 9.
Hernandez, E.G., Partida-Rodriguez, O., Camorlinga-Ponce, M., Nieves-Ramirez, M., Ramos-Vega, I., Torres, J., Perez-Rodriguez, M., 2018. Genotype B of Killer Cell Immunoglobulin-Like Receptor is Related with Gastric Cancer Lesions. Sci. Rep. 8, 6104.
Jamali, E., Barani, S., Yousefinejad, F., Ariafar, A., Talei, G.R., Ghaderi, A., 2018. KIRs gene content diversity in Iranians with urothelial bladder cancer. Mol. Biol. Rep. 45, 713.
Jiao, Y.-L., Zhang, B.-C., You, L., Li, J.-F., Zhang, J., Ma, C.-Y., Cui, B., Wang, L.-C., Chen, Z.-J., Zhao, Y.-R., 2010. Polymorphisms of KIR gene and HLA-C alleles: possible association with susceptibility to HLA-B27-positive patients with ankylosing spondylitis. J. Clin. Immunol. 30, 840.
Jones, S., Chen, W.-d., Parmigiani, G., Diehl, F., Beerenwinkel, N., Antal, T., Traulsen, A., Nowak, M.A., Siegel, C., Velculescu, V.E., 2008. Comparative lesion sequencing provides insights into tumor evolution. Proc. Natl. Acad. Sci. 105, 4283.
Khakoo, S.I., Rajalingam, R., Shum, B.P., Weidenbach, K., Flodin, L., Muir, D.G., Canavez, F., Cooper, S.L., Valiante, N.M., Lanier, L.L., 2000. Rapid evolution of NK cell receptor systems demonstrated by comparison of chimpanzees and humans. Immunity 12, 687.
Kim, H.-J., Choi, H.-B., Jang, J.-P., Baek, I.-C., Choi, E.-J., Park, M., Kim, T.-G., Oh, S.-T., 2014. HLA-Cw polypmorphism and killer cell immunoglobulin-like receptor (KIR) gene analysis in Korean colorectal cancer patients. Int. J. Surg. 12, 815.
Kovacic, M.B., Martin, M., Gao, X., Fuksenko, T., Chen, C.-J., Cheng, Y.-J., Chen, J.-Y., Apple, R., Hildesheim, A., Carrington, M., 2005. Variation of the killer cell immunoglobulin-like receptors and HLA-C genes in nasopharyngeal carcinoma. Cancer Epidemiology and Prevention Biomarkers 14, 2673.
Kuipers, E.J., Grady, W.M., Lieberman, D., Seufferlein, T., Sung, J.J., Boelens, P.G., van de Velde, C.J.H., Watanabe, T., 2015. Colorectal cancer. Nat. Rev. Dis. Primers 1, 15065.
Lasry, A., Zinger, A., Ben-Neriah, Y., 2016. Inflammatory networks underlying colorectal cancer. Nat. Immunol. 17, 230.
Levy, E.M., Roberti, M.P., Mordoh, J., 2011. Natural killer cells in human cancer: from biological functions to clinical applications. Biomed Res. Int. 2011.
Marcenaro, E., Pesce, S., Sivori, S., Carlomagno, S., Moretta, L., Moretta, A., 2013. KIR2DS1-dependent acquisition of CCR7 and migratory properties by human NK cells interacting with allogeneic HLA-C2+ DCs or T-cell blasts. Blood 121, 3396.
Martinez-Borra, J., Khakoo, S.I., 2008. Speed and selection in the evolution of killer-cell immunoglobulin-like receptors. Int. J. Immunogenet. 35, 89.
Mehta, R.S., Randolph, B., Daher, M., Rezvani, K., 2018. NK cell therapy for hematologic malignancies. Int. J. Hematol. 107, 262.
Middleton, D., Vilchez, J., Cabrera, T., Meenagh, A., Williams, F., Halfpenny, I., Maleno, I., Ruiz-Cabello, F., Lopez-Nevot, M., Garrido, F., 2007. Analysis of KIR gene frequencies in HLA class I characterised bladder, colorectal and laryngeal tumours. Tissue Antigens 69, 220.
Morales-Estevez, C., la Haba-Rodriguez, D., Manzanares-Martin, B., Porras-Quintela, I., Rodriguez-Ariza, A., Moreno-Vega, A., Ortiz-Morales, M.J., Gomez-España, M.A., Cano-Osuna, M.T., Lopez-Gonzalez, J., 2016. KIR genes and their ligands predict the response to anti-EGFR monoclonal antibodies in solid tumors. Front. Immunol. 7, 561.
Moretta, L., Moretta, A., 2004. Killer immunoglobulin-like receptors. Curr. Opin. Immunol. 16, 626.
Morris, E.J., Rutter, M.D., Finan, P.J., Thomas, J.D., Valori, R., 2015. Post-colonoscopy colorectal cancer (PCCRC) rates vary considerably depending on the method used to calculate them: a retrospective observational population-based study of PCCRC in the English National Health Service. Gut 64, 1248.
Munn, L.L., 2017. Cancer and inflammation. Wiley Interdiscip. Rev. Syst. Biol. Med. 9, e1370.
Ogino, S., Chan, A.T., Fuchs, C.S., Giovannucci, E., 2011. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. Gut 60, 397.
Olvera, A., Pérez-Álvarez, S., Ibarrondo, J., Ganoza, C., Lama, J.R., Lucchetti, A., Cate, S., Hildebrand, W., Bernard, N., Gomez, L., 2015. The HLA-C* 04: 01/KIR2DS4 gene combination and human leukocyte antigen alleles with high population frequency drive rate of HIV disease progression. AIDS 29, 507.
Al Omar, S.Y., Mansour, L., Dar, J.A., Alwasel, S., Alkhuriji, A., Arafah, M., Al Obeed, O., Christmas, S., 2015. The relationship between killer cell immunoglobulin-like receptors and HLA-C polymorphisms in colorectal cancer in a Saudi population. Genet. Test. Mol. Biomarkers 19, 617.
Ozturk, O.G., Gun, F.D., Polat, G., 2012. Killer cell immunoglobulin-like receptor genes in patients with breast cancer. Med. Oncol. 29, 511.
Popko, K., GórsKa, E., 2015. The role of natural killer cells in pathogenesis of autoimmune diseases. Cent. J. Immunol. 40, 470.
Pox, C.P., Altenhofen, L., Brenner, H., Theilmeier, A., Von Stillfried, D., Schmiegel, W., 2012. Efficacy of a nationwide screening colonoscopy program for colorectal cancer. Gastroenterology 142, 1460.
Rajalingam, R., 2011. Human diversity of killer cell immunoglobulin-like receptors and disease. Korean J. Hematol. 46, 216.
Rajalingam, R., 2018. Diversity of killer cell immunoglobulin-like receptors and disease. Clin. Lab. Med. 38, 637.
Rosenblum, M.D., Remedios, K.A., Abbas, A.K., 2015. Mechanisms of human autoimmunity. J. Clin. Invest. 125, 2228.
Saito, H., Hirayama, A., Umemura, T., Joshita, S., Mukawa, K., Suga, T., Tanaka, E., Ota, M., 2018. Association between KIR-HLA combination and ulcerative colitis and Crohn's disease in a Japanese population. PLoS One 13, e0195778.
Sugioka, D.K., Gonçalves, C.E.I., da Graça Bicalho, M., 2016. KIR repertory in patients with hematopoietic diseases and healthy family members. BMC Hematol. 16, 25.
Tian, Z., 2013. Frontiers in Natural Killer Cell Immunology. Nature Publishing Group.
Vilches, C., Castano, J., Gomez-Lozano, N., Estefania, E., 2007. Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. Tissue Antigens 70, 415.
Vogelstein, B., Fearon, E.R., Hamilton, S.R., Kern, S.E., Preisinger, A.C., Leppert, M., Smits, A.M., Bos, J.L., 1988. Genetic alterations during colorectal-tumor development. N. Engl. J. Med. 319, 525.
Yamagishi, H., Kuroda, H., Imai, Y., Hiraishi, H., 2016. Molecular pathogenesis of sporadic colorectal cancers. Chin. J. Cancer 35, 4.
Yan, X., Lu, J., Zou, X., Zhang, S., Cui, Y., Zhou, L., Liu, F., Shan, A., Lu, J., Zheng, M., 2018. The polypeptide N-acetylgalactosaminyltransferase 4 exhibits stage-dependent expression in colorectal cancer and affects tumorigenesis, invasion and differentiation. FEBS J.
Yu, H., Liu, F., Sansas, B., Kang, B., Preville, X., Wu, X., Chang, J., Micol, R., Wang, J., Meng, X., 2017. Typing of killer-cell immunoglobulin-like receptors and their cognate human leukocyte antigen class I ligands predicts survival of Chinese Han patients with metastatic non-small-cell lung cancer. Mol. Clin. Oncol. 6, 279.