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The association of the killer cell immunoglobulin-like receptor (KIR) genotype distribution and HLA-C ligands in colorectal cancer in the eastern region of Saudi Arabia

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ABSTRACT

Colorectal cancer (CRC) is the most common cause of cancer-related mortality globally. It ranks as the most common cancer among men and the third most prevalent among women in Saudi Arabia, especially in the eastern region. The immune system's response to cancer is significantly influenced by natural killer cells through their killer-cell immunoglobulin-like receptors (KIRs) interaction with human leukocyte antigen (HLA) molecules. Our research aimed to target a homogeneous ethnic group in the eastern region of Saudi Arabia, analyzing the distribution of 16 KIR genes and HLA-C1/C2 allotypes in 50 CRC patients and 63 healthy controls using the sequence-specific PCR amplification method. Findings revealed significantly lower frequencies of the 2DL2 and 3DL1 genes in CRC patients compared to controls (OR = 2.21; P = 0.04) and (OR = 7.46; P = 0.00009), respectively. Additionally, a higher prevalence of HLA-C2 was noted in CRC patients (84 %) versus controls (66.7 %) (OR = 2.26; P = 0.04). Conversely, the HLA-C1C2 combination showed a protective effect against CRC (OR = 0.38; P = 0.04). Our combinatorial analysis further identified specific gene combinations correlating with CRC risk or protection. Notably, the absence of KIR2DL2 combined with the presence of KIR2DS2 emerged as a significant risk factor, while various combinations involving KIR2DL2 and/or KIR2DS1 with HLA-C ligands were associated with either increased susceptibility or protection. The study underscores the protective role of KIR2DL2 against CRC in Saudi Arabia's eastern region and suggests that the absence of KIR2DL2, especially when KIR2DS2 is present, might increase CRC risk. This research contributes to understanding genetic influences on CRC susceptibility, emphasizing the potential of KIR and HLA interactions as biomarkers for CRC risk assessment.

1. Introduction

Colorectal cancer (CRC) stands as one of the most prevalent forms of cancer globally and is the primary reason for cancer-related fatalities worldwide. It ranks as the third most frequent cancer globally and was identified as the second major cause of cancer mortality in 2020 (Sung et al., 2021). In Saudi Arabia, CRC ranks as the second most prevalent form of cancer. It emerges as the foremost cancer affecting Saudi males, exhibiting an age-standardized rate (ASR) of 13.9 per 100,000, followed by non-Hodgkin's lymphoma and prostate cancer, with ASRs of 6.3 and 7.2 per 100,000, respectively. Among Saudi females, CRC stands as the third most prevalent cancer, with an ASR of 11.3 per 100,000, following breast cancer and thyroid cancer, which have ASRs of 33.7 and 13.7 per 100,000, respectively. Observations over recent decades indicate a continual rise in CRC incidence among the Saudi demographic (Chaudhri et al., 2020). Data from the Saudi Cancer Registry (SCR), established in 1994, underscore a significant increase in CRC incidence in the Kingdom of Saudi Arabia (KSA) (Ibrahim et al., 2008). Specifically, the ASR for CRC among Saudi males escalated from 9.9 in 2006 to 14.2 in 2018, whereas for Saudi females, the ASR rose from 8.8 in 2006 to 11.5 in 2018. From 2002 to 2017, there has been an increment in the annual number of diagnosed CRC cases among both genders in Saudi Arabia, with males consistently exhibiting higher ASRs than females. The cancer incidence reported in 2018 for Saudi Arabia highlighted

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significant regional disparities in colorectal cancer (CRC) incidence rates, as measured by Age-Standardized Rates (ASR). The Riyadh region exhibited the highest CRC incidence, with ASRs of 21.8 for males and 16.5 for females, surpassing the rates in the Eastern, Qassim, and Makkah regions. The Asir region displayed intermediate CRC incidence rates, with ASRs of 10.7 for males and 7.9 for females. Conversely, the Jouf region reported the lowest CRC incidence, with ASRs of 3.2 for males and 5.8 for females (Elwali et al., 2023).

The immune system's response to cancer is significantly modulated by the activity of natural killer cells (NK cells), as shown by Guillerey et al. (2016), through the interaction of their killer-cell immunoglobulin-like receptors (KIRs) with human leukocyte antigen (HLA) molecules (Parham & Guethlein, 2018). KIRs, also known as CD158, are a family of polymorphic transmembrane glycoproteins. They are mainly expressed on NK cells and a subset of T lymphocytes, playing an important role in regulating the development, activation, and tolerance of NK cells (Vilches & Parham, 2002; Caligiuri, 2008; Campbell & Purdy, 2011).

The KIR receptors display two or three extracellular domains and a cytoplasmic tail capable of transducing either activating or inhibitory signals. The nomenclature for KIR refers to the number of extracellular domains—KIR2D or KIR3D, respectively—and whether they have a long (L) inhibitory or a short (S) activating cytoplasmic tail (Long et al., 1996; Augusto et al., 2015). The most well-described ligands for KIR are the human leukocyte antigens (HLA) class I molecules (HLA-A, -B, or -C), which carry a selective range of epitopes, including Bw4, C1, or C2. Typically, KIR2D receptors recognize human leukocyte antigen-C (HLA-C) alleles, while KIR3D receptors recognize HLA-B or some HLA-A alleles (Graef et al., 2009; Béziat et al., 2017).

The KIR gene cluster is diversified by both gene content variability and allelic polymorphism and is located on the long arm of chromosome 19 (19q13.4) in a region called the leukocyte receptor complex (LRC) (Marsh et al., 2003; Solloch et al., 2020). The KIR gene family is comprised of 16 genes, classified as six genes encoding activating KIR (2DS1-5 and 3DS1), seven genes encoding inhibitory KIR (2DL1-3, 5 and 3DL1-2), KIR2DL4, which can show both inhibitory and activating function, and two pseudogenes (2DP1 and 3DP1). Furthermore, KIR3DL3, KIR3DP1, KIR2DL4, and KIR3DL2 are framework genes and are always present in the genome (Rajalingam, 2011; Chou et al., 2020).

KIR haplotypes can be divided into two major classes, A and B haplotypes. KIR haplotypes have variable genes number; however, the framework genes are present in virtually all haplotypes (Li et al., 2008). The A haplotype includes four genes in addition to the framework genes (KIR2DL1, KIR2DL3, KIR3DL1, KIR2DS4), and encodes for mostly inhibitory receptors (Wilson et al., 2000). B haplotypes, on the other hand, have variable gene content and contain several genes (2DS1, 2DS2, 2DS3, 2DS5, 2DL2, 2DL5, and 3DS1) that are different to the A haplotype, and, therefore, B haplotypes encode more activating KIRs compared with the A haplotype (Middleton et al., 2007; Rajalingam, 2011). Not all KIR genes exist in all individuals; this unusual presence/absence polymorphism creates a wide variety of haplotypes that vary between individuals and populations. The increased number of alleles in every locus combined with diversity of haplotypes make it mostly impossible for two unrelated individuals to have the same KIR genotype, which generates extraordinary KIR diversity in humans (Augusto, 2016).

Several studies have shown a relation among KIR receptors and their ligand and the protection or susceptibility of solid tumors (Dębska-Zielkowska et al., 2021; Jobim et al., 2013). Previous studies have reported associations between certain KIR/HLA compound genotypes and colorectal cancer risk (Al Omar et al., 2015; Canossi et al., 2016). Additionally, certain activating KIRs indicate a genotype that showed resistance to tumor recurrence in the presence of their ligands (Beksac et al., 2015).

To confirm any potential involvement of allelic combinations in the KIR and HLA genes in CRC risk, much bigger investigations in ethnically homogeneous populations are required. In the present study, in order to reduce the probability of high rates of false positives caused by population stratification, we examined ethnically homogeneous case and controls and assessed how the diversity of KIR genotypes and their HLA-C ligands affected the risk of CRC, with a specific emphasis on the eastern region of Saudi Arabia.

2. Materials and methods

2.1. Subjects

A case-control study was performed on 113 eligible subjects from the eastern region of Saudi Arabia. Peripheral blood samples were obtained from 50 Saudi patients (mean age 55 ± 13.5 years) diagnosed with CRC, and 63 Saudi healthy controls (mean age 38.8 ± 15 years). All participants gave informed consent and agreed to provide blood samples for this study.

	Patients mean ± STD	Control mean ± STD
Number of subjects	50	63
Age (yrs)	55 ± 13.5	38.8 ± 15

2.2. Genomic DNA extraction and KIR, HLA-C typing

Genomic DNA was extracted from peripheral blood using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA), according to the manufacturer's recommendation. DNA was quantitated for each sample by using a NanoDrop 8000 (Thermo Scientific®, USA).

Genotyping of KIRs was performed by using the polymerase chain reaction (PCR)-sequence-specific primer PCR-SSP approach, for the 16 common KIR genes. For HLA-C1 and HLA-C2 typing, the same primers were used as described by Tajik et al. (2010), which enables the detection of all known KIR genes.

The thermocycler T100TM (Bio-Rad, United States) was used for all PCR reactions. The PCR products were examined for the presence or absence of gene-specific amplicons by electrophoresis in 2 % agarose gels stained with ethidium bromide and viewed on a UV transilluminator using a gel documentation system (BioRad Gel225 DocXR +).

2.3. Statistical analysis

Direct counting was used to determine the frequency of each KIR, HLA-C ligand, and KIR/HLA-C ligand combination in the patient and control groups. Multiple logistic regression models were performed to estimate odds ratios (ORs), confidence intervals (CIs) at 95 % and P value. When the genotype and allele numbers were less than 5, the Fisher exact test was used. SNPstats and SigmaPlot version 11 software were used for data analysis (Sole et al., 2006). Hardy–Weinberg equilibrium (HWE) for both the patient and control groups was estimated using SNPStats. The statistical significance was defined as p less than 0.05. For multiple comparisons, the Bonferroni correction was applied. To choose the best fitted model, the Akaike information criterion (AIC) was used.

3. Results

In this case–control study, we examined the distribution of 16 KIR genes, HLA-C1/C2 allotypes for 50 CRC patients and 63 healthy controls using sequence-specific PCR amplification method. The framework genes were present in both patients and control groups. Significantly lower frequencies for the 2DL2 and 3DL1 genes were observed in the CRC group compared with the control group (OR = 2.21; P = 0.04) and (OR = 7.46; P = 0.00009), respectively. Regarding the rest of the genes, there were no significant variations between the groups (Table 1).

Table 1
Comparison of KIR gene frequencies between CRC patients and healthy control groups.

Genes	Patients freq%		Controls freq %		OR	X^2	CI 95 %	P-value
3DS1	15	30 %	13	20.6 %	0.60	1.31	0.25—1.43	0.25
2DL1	49	98 %	55	87.3 %	0.14	4.35	0.01—1.16	0.07
3DL3	50	100 %	63	100 %	–	–	–	–
2DL2	22	44 %	40	63.5 %	2.21	4.28	1.03—-4.72	0.04
3DL1	28	56 %	57	90.5 %	7.46	17.78	2.71—20.48	0.00009
2DS4	45	90 %	58	92.1 %	–	0.15	–	–
2DL3	42	84 %	50	79.4 %	0.73	0.40	0.27—1.93	0.53
2DL5	34	68 %	35	55.6 %	0.58	1.82	0.27—1.27	0.18
2DL4	50	100 %	63	100 %	–	–	–	–
2DS3	22	44 %	27	42.9 %	0.95	0.01	0.45—2.01	0.90
2DP1	50	100 %	63	100 %	–	–	–	–
2DS5	17	34 %	17	27 %	0.71	0.65	0.32—1.60	0.42
2DS2	30	60 %	35	55.6 %	0.83	0.23	0.39—1.76	0.63
3DL2	50	100 %	63	100 %	–	–	–	–
3DP1	50	100 %	63	100 %	–	–	–	–
2DS1	13	26 %	21	33.3 %	1.42	0.71	0.62—3.23	0.4

Na: Non-attributed; OR: Odds Ratio; CI: Confidence interval. Significant associations are shown in bold.

The frequency of the main ligands of KIR receptors, HLA-C allotypes, was also investigated. The frequency of the two major allotypes, HLA-C1 and C2, were analyzed in both CRC patients and healthy controls. Statistical analysis showed a significant increase of the –C2 group in CRC patients (84 %) as compared with controls (66.7 %()OR = 2.26; 95 % CI = 1.04—-6.58; p = 0.04). Furthermore, genotype distribution demonstrated that HLA-C1C2 was protective against CRC (OR = 0.38; 95 % CI = 0.15–0.95, and p = 0.04) (Table 2).

Different gene combinations were examined to determine the relative contribution of effective genes involved in CRC development. Analyses showed that KIR2DS2 could be a risk factor significantly in cases of the absence of KIR2DL2 (OR = 0.13; p = 0.004), whereas no significant difference was observed in the presence of KIR2DL2 and in the absence of KIR2DS2. For the rest of the genes, no significant differences between the CRC group and the healthy control group were detected (Table 3).

In this study, we also analyzed the combined association of KIR genes and HLA-C ligands, C1-C2 with both CRC patients and healthy control groups. Remarkably, for inhibitory genes, the combinations KIR2DL1 +/HLA-C2 + and KIR2DL1+/HLA-C1C2 + showed a significant positive correlation with CRC (OR = 0.33; and p = 0.01) and (OR = 0.46; p = 0.04), respectively. A significant negative correlation with CRC was also observed for the combinations KIR2DL2+/HLA-C1C1+ (OR = 4.47; p = 0.01) and KIR2DL2/3+/HLA-C1+ (OR = 2.62; p = 0.04). For the activating genes, these exhibited a significant positive association with CRC for the combination KIR2DL2-/KIR2DS2+/HLA-C1+ (OR = 0.09; p = 0.01). A negative association with CRC was detected for the combinations KIR2DS1+/HLA-C2- (OR = 8.16; p = 0.02) and KIR2DS1+/HLA-C1C1+ (OR = 8.16; p = 0.02) (Table 4).

Table 2
The frequencies of KIR ligands C1-C2 in CRC patients and healthy control groups.

Gene/ genotype	Patients freq%	Controls freq %	OR	X^2	CI 95 %	P-value
C1	33 (66 %)	44 (69.8 %)	0.83	0.19	0.37—1.85	0.66
C2	42 (84 %)	42 (66.7 %)	2.62	4.39	1.04—6.58	0.04
C1C2	8 (16 %)	21 (33.3 %)	0.38	4.39	0.15—0.95	0.04
C1C1	17 (34 %)	19 (30.2 %)	1.73	0.19	0.81—3.70	0.15
C2C2	25 (50 %)	23 (36.5 %)	1.19	2.08	0.53—2.64	0.66

OR: Odds Ratio; CI: Confidence interval. Significant associations are shown in bold.

Table 3
Combinatorial analyses of selected KIR receptors among CRC patient and control groups.

KIR/ Ligand	Patients/ freq%		Controls/freq %		OR	X^2	P-value
2DS2+/2DS3+	17	34 %	23	36.5 %	1.11	0.08	0.78
2DS2+/2DS3-	13	26 %	12	19 %	0.67	0.78	0.37
2DS2-/2DS3+	5	10 %	4	6.3 %	0.61	0.51	0.47
2DS2-/2DS3-	15	30 %	24	38.1 %	1.43	0.81	0.36
2DS2+/2DS3+/ 2DL5+	17	34 %	22	34.9 %	1.04	0.01	0.91
2DS2+/2DS3+/2DL5-	0	0 %	1	1.6 %	–	–	0.37
2DS2+/2DS3-/2DL5+	9	18 %	5	7.9 %	0.39	2.60	0.10
2DS2-/2DS3+/2DL5+	5	10 %	2	3.2 %	0.29	2.23	0.13
2DS2-/2DS3+/2DL5-	0	0 %	2	3.2 %	–	–	0.20
2DS2+/2DS3-/2DL5-	4	8 %	7	11.1 %	1.43	0.31	0.58
2DS2-/2DS3-/2DL5+	3	6 %	6	9.5 %	1.64	0.47	0.49
2DS2-/2DS3-/2DL5-	12	24 %	18	28.6 %	1.26	0.30	0.58
2DL2+/2DS2+	20	40 %	33	52.4 %	1.65	1.72	0.19
2DL2+/2DS2-	2	4 %	7	11.1 %	3	1.92	0.16
2DL2-/2DS2+	10	20 %	2	3.2 %	0.13	8.31	0.004
2DL2-/2DS2-	18	36 %	21	33.3 %	0.88	0.09	0.76

OR: Odds Ratio; Significant associations are shown in bold.

4. Discussion

To the best of our knowledge, this is the first study that has analyzed the relationship between the distributions of KIR genes and HLA-C ligands and the development of CRC in the eastern region of Saudi Arabia. Two inhibitory KIR genes, 2DL2 and 3DL1, were identified to be negatively correlated to CRC. These results suggest that inhibitory 2DL2 and 3DL1 might have protective effects against CRC. Kim et al. (2014) reported that higher frequencies of 3DL1, 2DS2, and 2DS4 showed protective effects against CRC. Similar study revealed that KIR3DL1, KIR3DS1, KIR2DS1, and KIR2DS4 have a protective role against metastasis in patients with colorectal adenocarcinoma (Barani et al., 2019). Both studies conform to our results regarding the 3DL1 gene. However, a recent study (Hematian Larki et al., 2022) conflicts with our results and reported that 2DL2 and 2DS2 genes conferred a high risk of developing lung cancer in the native population of southern Iran. Similar study in a Brazilian population showed that the KIR2DL2 gene is positively related with the development of breast cancer (Jobim et al., 2013). In a more recent study, the KIR2DS3 gene was determined as positively associated with the development of CRC (Diaz-Peña et al.,

Table 4
The distribution of the frequencies of KIR genes in presence and absence of their C1-C2 ligands between CRC patients and healthy control groups.

KIR/ Ligand	Patients/ freq%		Controls/freq %		OR	X^2	P-value
2DL2 + C1	16	32 %	29	46 %	1.81	2.29	0.13
2DL2 + C1-	6	12 %	11	17.5 %	1.55	0.65	0.42
2DL2 + C1C1+	3	6 %	14	22.2 %	4.47	5.74	0.01
2DL2 + C1C2+	13	26 %	15	23.8 %	0.88	0.07	0.78
2DL3 + C1+	25	50 %	34	54 %	1.17	0.18	0.67
2DL3 + C1−	17	34 %	16	25.4 %	0.66	1.00	0.31
2DL3 + C1C1+	8	16 %	16	25.4 %	1.78	1.47	0.22
2DL3 + C1C2+	17	34 %	18	28.6 %	0.77	0.38	0.53
2DL2/3 + C1+	8	16 %	21	33.3 %	2.62	4.39	0.04
2DL2/3 + C1−	6	12 %	8	12.7 %	1.06	0.01	0.91
2DL2/3 + C1C1+	3	6 %	10	15.9 %	2.95	2.67	0.10
2DL2/3 + C1C2+	5	10 %	11	17.5 %	1.90	1.28	0.25
2DS2 + C1+	21	42 %	27	42.9 %	1.03	0.01	0.92
2DS2 + C1−	9	18 %	8	12.7 %	0.66	0.61	0.43
2DS2 + C1C1+	4	8 %	12	19 %	2.70	2.80	0.09
2DS2 + C1C2+	17	34 %	15	23.8 %	0.60	1.43	0.23
2DL1 + C2+	41	82 %	38	60.3 %	0.33	6.23	0.01
2DL1 + C2−	8	16 %	17	27 %	1.94	1.95	0.16
2DL1 + C2C2+	16	32 %	18	28.6 %	0.85	0.16	0.69
2DL1 + C1C2+	25	50 %	20	31.7 %	0.46	3.88	0.04
2DS1 + C2+	12	24 %	12	19 %	0.74	0.41	0.52
2DS1 + C2–	1	2 %	9	14.3 %	8.16	5.22	0.02
2DS1 + C1C1+	1	2 %	9	14.3 %	8.16	5.22	0.02
2DS1 + C1C2+	9	18 %	7	11.1 %	0.5	1.09	0.29
2DS1 + C2C2+	3	6 %	5	7.9 %	1.35	0.16	0.69
2DL2+/2DS2+/C1+	14	28 %	26	41.3 %	1.80	2.15	0.14
2DL2+/2DS2-/C1+	2	4 %	3	4.8 %	1.2	0.04	0.84
2DL2-/2DS2+/C1+	7	14 %	1	1.6 %	0.09	6.53	0.01
2DL2+/2DS2+/C1-	6	12 %	7	11.1 %	0.91	0.02	0.88
2DL2-/2DS2-/C1+	10	20 %	14	22.2 %	1.14	0.08	0.77

OR: Odds Ratio; Significant associations are shown in bold.

2020). The KIR2DS3 gene was neutral in our study; this might be due to the small sample size of our study, which could affect the results.

Contrary to above mentioned studies, no correlation was observed between KIRs and CRC in Europeans (Middleton et al., 2007). Furthermore, study on patients with different solid tumors (including those with non-small-cell lung cancer, small cell lung cancer, colon cancer, and kidney cancer patients), showed no statistically significant differences in KIR genes in comparison to the control group (Al Omar et al., 2010). Also, no significant differences were found between KIR genes and CRC in a Brazilian Caucasoid population (Portela et al., 2017).

Regarding the HLA-C ligand, we found a significant positive correlation between the HLA-C2 allotype and the risk of CRC, as reported on kidney cancer patients (Al Omar et al., 2010). On the contrary, we recognized a protective effect with the heterozygote genotype HLA-C1C2. This differed from those reported in studies on breast cancer in Saudi Arabia and Brazilian Caucasian populations, which showed a positive relationship between HLA-C1C2 and the occurrence of breast cancer (Jobim et al., 2013; Alomar et al., 2017). This might be explained by variations in the immune mechanisms involved in the development of breast and colorectal cancer.

In addition, we noticed an additional effect following analysis of possible gene combinations linked with CRC. The activating gene KIR2DS2 might play a role in CRC development in the absence of inhibitory gene KIR2DL2. In contrast, no protective effect was observed against CRC in the presence of KIR2DL2 and in the absence of KIR2DS2. Contrarily, Alomar et al. (2017) suggested that the KIR2DS2 gene has a protective effect against breast cancer in the absence of KIR2DL2.

When considering the interaction between some KIR receptors and their unique HLA-C ligands C1 and C2 groups, our results showed an association with an elevation of the frequency of KIR2DL1-C2 and CRC, individually or heterozygote. In a study in Turkey two groups of CRC patients underwent resection surgery. Group 1 manifested metastatic cancer recurrence during the study period while Group 2 patients remained disease-free during the study period. They found that KIR2DL1-C2 was related with CRC recurrence (Beksac et al., 2015). Conversely, KIR2DL2 and HLA-C showed a protective effect in individuals sharing the KIR2DL2 gene and the HLA-C1 ligand at homozygote status which is in accordance with kidney cancer findings (Al Omar et al., 2010). The protection effect against CRC was confirmed when KIR2DL2 and KIR2DL3 were accumulated together with the C1. It is worth noting that 2DL2 was at first suspected to be negatively related to the CRC development. It is interesting to note that KIR2DL2/3 and its C1 had the opposite effect on the development of CRC, which is conflicting with our findings (Al Omar et al., 2015). However, we found a strong positive association of KIR2DS2 in the absence of KIR2DL2 with risk of CRC. This association was confirmed when considering the presence of C1 ligand. These results differed from that reported in a study on the Korean population, but we must mention that they did not examine in the case of the absence of KIR2DL2 (Kim et al., 2014).

In conclusion, the present study highlights the major role of KIR2DL2 in protection against CRC in the eastern region of Saudi Arabia, and the opposite role given the absence of KIR2DL2 in the presence of KIR2DS2.

In future study, we will examine the expression level and the functional study of KIR2DL2 in different types of immune cells such as NK cells and CD8 T cells. Furthermore, to confirm our results we recommend using a larger sample size in the same ethnically homogeneous case and controls.

CRediT authorship contribution statement

Sarah Alqadheeb: Writing – original draft, Software, Methodology, Formal analysis. **Afrah Alkhuriji:** Writing – review & editing. **Fadwa M. Alkhulaifi:** Writing – review & editing, Validation, Formal analysis. **Hussah M. Alobaid:** Writing – review & editing. **Rasha Alonaizan:** Writing – original draft. **Suliman Alomar:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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