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ORIGINAL ARTICLE

# *KIRs* gene content diversity in Iranians with urothelial bladder cancer

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**Abstract**
Natural killer cells (NK) are the first arm of the innate immune system in defense against tumor and infection. 16 distinct Killer-cell immunoglobulin-like receptors (*KIRs*) are involved in orchestrating NK cell function. The KIR family contains 14 genes and 2 pseudogenes. Six of these receptors are activating (aKIR) and the remaining receptors are inhibitory KIRs (iKIR), that interact with MHC-I molecules; producing signals which stop NK cell function. In the current study, we have investigated the genomic diversity of *KIRs* and determining the A and B haplotypes as well as Bx subsets in 119 patients with bladder cancer and 200 healthy controls to find out if there is an association between KIR system and susceptibility to bladder cancer. Polymerase chain reaction with sequence specific primers (SSP–PCR) typing system was used to determine the *KIR* gene profile. The results implicated decreased frequency of inhibitory *KIR2DL2* and activating *KIR2DS2* while increased frequency of CxT4 genotypes in patients compared with healthy controls. Among Bx subsets, the CxT4 gene cluster is more frequent in bladder cancer patients compared to controls. Our results provide a conclusion that *KIR2S2* and *KIR2L2* may play a protective role against bladder cancer development while the CxT4 gene cluster may underlie susceptibility to bladder cancer in Iranian population.

**Keywords** Killer immunoglobulin like receptors (KIRs) · Bladder cancer · Natural killer cell · Polymerase chain reaction with sequence specific primers (PCR–SSP)

## Introduction

Urothelial bladder cancer (UBC) is the most common malignancy of urinary system [1, 2]. It has high morbidity and mortality rate, as well as high rate of recurrence to invasive forms [2]. The disease incidence in men is three times more than women. A combination of environmental and genetic factors affect susceptibility to UBC. Tobacco smoking, age, and occupational exposure to polycyclic hydrocarbons like aromatic amines are most important risk factors for UBC [3–5]. Along with the development of the molecular immunology, the association of immune response genes and various diseases has been highlighted. Human leukocyte antigen (HLA) and Killer-cell immunoglobulin-like receptor (KIR) genotyping studies has been a field of interest; is being extensively typed in many diseases [6, 7].

*KIRs* are one of the immune response genes which play crucial role in NK cell mediated functions. KIR molecules are a polymorphic family of receptors encoded by a gene cluster on 19 q13.4. 14 distinct *KIR* genes and 2 pseudogenes have been identified. The *KIR2DL1, 2DL2, 2DL3, 2DL5, 3DL1, 3DL2*, and *3DL3* have an inhibitory function; containing immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic tail. *KIR2DS1, 2DS2, 2DS3, 2DS4, 2DS5*, and *3DS1* are activating *KIRs. 2DP1* and *3DP1* are known as pseudogenes [7]. The net balance of signal from activating and inhibitory receptors will determine the NK cell final destiny. According to *KIR* gene content two distinct haplotypes has been introduced. Haplotype A containing *KIR2DL3, 2DP1, 2DL1, 3DL1, 2DS4*, and haplotype B which is more variable and includes diverse combinations of *KIR2DS1, 2DS2, 2DS3, 2DS5, 2DL2, 2DL5*, and 3DS1. *KIR2DL4, 3DL2, 3DL3*, and *3DP1* are as framework genes

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which present on both haplotypes. Inhibitory *KIRs* recognize HLA I molecules. KIR2DL2 and KIR2DL3 recognize HLAC1 (HLAC with Asp at position 80), *KIR2DL1* bind HLAC2 (HLAC with Lys at position 80), KIR3DL2 ligates with HLAA3/A11, and KIR3DL1 binds HLABw4 (HLA B allotype containing Bw4 epitope). The ligands for activating KIR receptors are mostly unknown, but some of them can recognize HLA class I with strongly lower affinity than inhibitory ones [8–10].

Due to different haplotype inheritance patterns of KIR from parents, as well as the allelic polymorphism of *KIR* genes, the *KIR* gene cluster indicates extensive diversity. This diversity may affect susceptibility or resistance to different diseases. *KIR* genotyping studies have been done in autoimmune diseases, cancers, hematologic malignancies, etc. [11]. Such studies has not been extensively done in UBC patients. According to our information, only one KIR genotyping study with limited sample size (n = 65) has been done by Middleton et al. on UBC patients. According to this study *KIR2DS4* and *KIR3DL1* are more frequent in UBC patients than healthy normal controls [12].

The aim of this study is to find out the diversity of *KIR* genes, genotypes and haplotypes and their comparison between UBC patients and healthy controls.

## Material and method

### Study subjects

119 patients with pathological confirmed bladder cancer (mean age: 65.23, 83% male and about 17% female) and 200 healthy controls were included in this study. The cases were recruited at Namazi Hospital and controls from Motahari Clinic. Patients and healthy controls were matched by age (± 5 years) and sex (demographic statistics are shown in Table 1). Both groups were ethnically matched and were from the Fars province in the southern part of Iran. Furthermore, bladder cancer patients were categorized to advanced and early stage, according to the TNM staging system. The study was also reviewed and approved by the Medical Research Ethics Committee of Shiraz University of Medical Sciences (EC-9372-7202).

**Table 1** Demographic characteristics of cases and controls

| Group | Bladder cancer n = 119 | Controls n = 200 |
|---|---|---|
| Mean of age ± SD | 65.23 ± 21.10 | 61.6 ± 14.03 |
| %Female (N) | 16.8 (20) | 19.0 (38) |
| %Male (N) | 83.1 (99) | 81.0 (162) |

### KIR genotyping

Genomic DNA from all the subjects was extracted from whole blood samples using a QIAamp DNA Mini Kit (Qiagen, Germany). PCR-SSP method was used to characterize the presence/absence of 16 *KIR* genes in UBC patients and healthy controls. 100 ng of genomic DNA was amplified in 7.5 µl Taq DNA polymerase 1× Master Mix RED. Specific primers designed by Vilches et al. were used to detect specific *KIR* genes [13] and primers designed by Ashouri et al. [14] for segregation of *KIR2DS4* full and deleted variants. In addition to specific primers three pair of internal controls were used to amplify 16 *KIR* genes in range of 0.4–2.6 µM in specific primers and 0.1 µM in internal controls [13].

PCR reactions were performed in the ABI thermal cycler according the following thermal condition: initial denaturation for 2 min at 95 °C, then 10 cycles genes of 10 s at 94 °C and 40 s at 65 °C; and 20 cycles of 20 s at 94 °C, 20 s at 61 °C and 30 s at 72 °C for final extension [13]. UCLA reference DNA with known KIR genotype, provided by Professor Rajalingam and co-workers was used to check the accuracy and specificity of the method [14]. KIR typing was done by detecting known PCR product size by comparing with DNA ladder (50 bp). Finally, we typed 11 unique genotypes again to validate the accuracy of data.

### Data analysis and statistical methods

We determined the frequency of each KIR gene, A and B haplotypes, Bx subtypes as well as KIR gene clusters and compared them between patients and control group by two-tailed Chi square test of association in *Graphpad prism software* and $p < 0.05$ was considered to be statistically significant. The significant results were adjusted by Yates' correction. The KIR haplotypes based on gene content divided to Group A haplotypes with specified gene contents (*KIR3DL3-2DL3-2DP1-2DL1-3DP1-2DL4-3DL1-2DS4-3DL2*) and group B haplotypes with variable gene contents (*KIR2DL2-2DL5-2DS1-2DS2-2DS3-2DS5-3DS1*) which did not occur in A haplotype [15]. In the KIR gene complex two frequently occurring gene clusters are introduced due to the linkage disequilibrium, the C4 cluster with *KIR2DS2-2DL2-2DS3-2DL5* genes is located at the centromeric half and T4 cluster with *KIR3DS1-2DL5-2DS1-2DS5* genes is located at the telomeric half of the complex. So the Bx genotype will be divided into four subtypes C4Tx, CxT4, C4T4, and CxTx.

## Results

### Comparison of *KIR* genes frequency in bladder cancer patients and healthy controls

To determine the *KIRs* role as a genetic risk for bladder cancer, we studied the presence/absence of 16 different *KIR* genes and *KIR2DS4* variants in 119 bladder cancer patients and 200 healthy controls. The distribution of *KIR* genes in patients with bladder cancer and healthy controls is illustrated in Table 2. The framework genes *KIR2DL4, 3DL2, 3DL3* and *3DP1* were present in all 319 individuals.

We observed that A haplotypes associated KIR genes (*2DL1 2DL3 3DL1 2DS4)* had higher frequency (> 89.5%) than B haplotypes associated ones both in cases and controls. *KIR2DL1* (99.1%) and *KIR2DS4* (91.5%) were the highest while the *KIR2DL2* (53.7%) and *KIR2DS* (36.9%) were the lowest inhibitory and activating KIR genes respectively in bladder cancer patients. We found a highly significant decrease in the carrier frequency of the inhibitory *KIR2DL2* (53 vs. 69% p value: 0.00690, OR 1.9, CI 1.2–3.1) and activating *KIR2DS2* (52 vs. 69%, p value: 0.0037, OR 2.046, CI 1.2–3.2) in bladder cancer patients compared to controls (Table 2).

To comparison the variants of KIR2DS4 between cases and controls, the individuals were categorized into three groups: (A) Del: individuals who had only the deletion variant of KIR2DS4 (B) Full: individuals who had only the full-length KIR2DS4, and (C) Full/Dell: individuals who had both full-length and deletion variant of KIR2DS4 together. However we found an increase in the carrier frequency of KIR2DS4 Del in bladder cancer (76.1%) in comparison to CNs (72.0%) but there was no significant difference (Table 3).

### Comparison of KIR haplotype frequency in bladder cancer patients and healthy controls

The frequency of AA genotype carriers was higher (26.8 vs. 21.5%) while the Bx genotype carrier frequency was lower (73.2 vs. 78.5) in patients with bladder cancer compared with healthy controls, however there were no significant difference between them. Furthermore, the difference between the frequency of A and B haplogroup between patients and control group was not statistically significant (data are shown in Table 4).

**Table 2** Frequency of KIR genes in bladder cancer patients and healthy controls

| KIR gene | Bladder cancer n = 119 | | Controls n = 200 | | Bladder cancer versus controls | | |
|---|---|---|---|---|---|---|---|
| | %F | (N+) | %F | (N+) | *P* value | Odds ratio | 95% CI |
| A haplotypes associated *KIR* genes | | | | | | | |
| *2DL1* | 99.1 | (118) | 99.5 | (199) | > 0.9 | 0.59 | 0.03–11.3 |
| *2DL3* | 93.2 | (111) | 89.5 | (179) | 0.3 | 1.62 | 0.71–3.5 |
| *3DL1* | 91.5 | (109) | 96.0 | (192) | 0.130 | 0.45 | 0.17–1.22 |
| *2DS4* | 91.5 | (109) | 96.5 | (193) | 0.070 | 0.39 | 0.15–1.11 |
| B haplotypes associated *KIR* genes | | | | | | | |
| *2DL2* | 53.7 | (64) | 69.5 | (139) | **0.0069*** | **1.9** | **1.2–3.1** |
| *2DL5* | 62.1 | (74) | 61.0 | (122) | 0.9 | 1.05 | 0.65–1.67 |
| *3DS1* | 42.0 | (50) | 33.5 | (67) | 0.14 | 1.43 | 0.9–2.2 |
| *2DS1* | 42.0 | (50) | 43.5 | (87) | 0.81 | 0.94 | 0.6–1.49 |
| *2DS2* | 52.1 | (62) | 69.0 | (138) | **0.0037*** | **2.046** | **1.2–3.2** |
| *2DS3* | 42.0 | (50) | 49.5 | (99) | 0.2 | 0.73 | 0.47–1.16 |
| *2DS5* | 36.9 | (44) | 29.0 | (58) | 0.17 | 1.43 | 0.87–2.32 |
| Framework genes/pseudogenes | | | | | | | |
| *2DL4* | 100 | (119) | 100 | (200) | | | |
| *3DL2* | 100 | (119) | 100 | (200) | | | |
| *3DL3* | 100 | (119) | 100 | (200) | | | |
| *2DP1* | 99 | (118) | 99.5 | (199) | > 0.9 | 0.59 | 0.03–11.3 |
| *3DP1* | 100 | (119) | 100 | (200) | | | |

Frequency of KIR genes expressed as percentage and defined as the number of individuals with KIR genes (N+) divide by number of individuals studies in the given study group (n). *Con* controls, (*) significant p value Yates correction

## Comparison of carrier frequency of Bx subtypes in bladder cancer patients and healthy controls

The frequency of Bx subtypes was determined and the analysis of the results indicates that the CxT4 genotypes carriers was significantly higher in patients compared to controls (20 vs. 8.5%; p-value: 0.004, OR 2.76, CI 1.43–5.44). In addition, C4 gene cluster containing *KIR2DS2-2DL2-2DS3-2DL5* is more frequent in the control group compared to controls (p-value: 0.03, OR 0.59, CI 0.36–0.94). The distribution of Bx subtypes in patients with bladder cancer and healthy controls is illustrated in Table 5.

## KIR genotypes diversity in the study population

34 distinct genotypes were found among 319 individuals, which is illustrated in Table 6. These genotypes were defined by referring to the allele frequency database (http://www.allelefrequencies.net). We found 11 unique genotypes which

**Table 3** Distribution of *KIR2DS4* variants in bladder cancer patients and healthy controls

| KIR2DS4 variants | Bladder cancer n = 109 | Control n = 193 | Bladder cancer versus controls | | |
|---|---|---|---|---|---|
| | %F (N) | %F (N) | *p* Value | Odds ratio | 95% CI |
| Del | 76.1 (83) | 72.0 (139) | 0.49 | 1.24 | 0.71–2.09 |
| Full | 16.5 (18) | 22.2 (43) | 0.29 | 0.69 | 0.38–1.24 |
| Full/Dell | 6.4 (7) | 5.1 (10) | 0.79 | 1.25 | 0.48–3.54 |
| Missing | 0.9 (1) | 0.4 (1) | | | |

The number (n) that is exhibited below each group is the number of people who were 2DS4 positive in each group that is categorized to three types (Deletion, Full and Full/Deletion) of *2DS4* gene. Two tailed Fisher exact probability (p) test, odd ratio with 95% CI was calculated by Graph pad prism software and $p < 0.05$ was considered to be statistically significant

**Table 4** Frequency of KIR genotypes and haplotypes in Bladder cancer patients and healthy controls

| | Bladder cancer n = 119 | | Controls n = 200 | | Bladder cancer versus controls | | |
|---|---|---|---|---|---|---|---|
| | %F | (N+) | %F | (N+) | *p* Value | Odds ratio | 95% CI |
| Genotype | | | | | | | |
| AA | 26.8 | (32) | 21.5 | (43) | 0.27 | 1.34 | 0.79–2.29 |
| Bx | 73.2 | (87) | 78.5 | (157) | | | |
| Haplotype | | | | | | | |
| A | 55.8 | (133) | 53.9 | (217) | 0.43 | 0.74 | 0.35–1.56 |
| B | 44.1 | (105) | 46.0 | (185) | 0.21 | 0.61 | 0.28–1.30 |

Frequency of KIR genotypes/haplotypes presented as percentage and defined as the number of individuals with genotype/haplotype (N+) divided by number of individuals studies in the given study group (n). *Con* controls

**Table 5** Frequency of Bx subtypes in bladder cancer patients and healthy controls

| KIR genotypes/clusters | Bladder cancer n = 119 | | Controls n = 200 | | Bladder cancer versus controls | | |
|---|---|---|---|---|---|---|---|
| | %F | (N+) | %F | (N+) | *p* value | Odds ratio | 95% CI |
| C4Tx genotype | 21.0 | (25) | 33.0 | (66) | 0.052 | 0.55 | 0.31–0.96 |
| CxT4 genotype | 20.0 | (24) | 8.5 | (19) | **0.0114*** | **2.76** | **1.43–5.44** |
| C4T4 genotype | 14.2 | (17) | 15.0 | (30) | > 0.9 | 1.02 | 0.51–1.09 |
| CxTx genotype | 17.6 | (21) | 21.0 | (42) | 0.7600 | 0.87 | 0.47–1.57 |
| C4 gene cluster | 35.2 | (42) | 48.0 | (96) | **0.0359*** | **0.59** | **0.36–0.94** |
| T4 gene cluster | 34.4 | (41) | 24.5 | (49) | 0.07 | 1.62 | 0.99–2.62 |

Two tailed Fisher exact probability (p) test was calculated by Graph pad prism software and *P < 0.05: statistically significant; based on two-tailed Chi square with Yates' correction

No significant P value was found

**Table 6** KIR genotypes distribution in the study population

| # Genotype | KIR ID | Group | 3DL1 | 2DL1 | 2DL3 | 2DS4 | 2DL2 | 2DL5 | 3DS1 | 2DS1 | 2DS2 | 2DS3 | 2DS5 | 2DL4 | 3DL2 | 3DL3 | 2DP1 | 3DP1 | Bladder cancer n=119 | | Control n=200 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | F% | (N) | F% | (N) |
| 1 | 1 | AA | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ | ■ | ■ | ■ | ■ | ■ | | | 0.5 | 1 |
| 2 | 14 | Bx | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | ■ | ■ | ■ | ■ | ■ | 26 | 31 | 21 | 42 |
| 3 | 23 | | ■ | □ | □ | ■ | ■ | □ | □ | □ | ■ | □ | □ | ■ | ■ | ■ | ■ | ■ | | | 0.5 | 1 |
| 4 | 200 | | ■ | □ | □ | ■ | ■ | ■ | □ | ■ | ■ | ■ | □ | ■ | ■ | ■ | □ | ■ | 0.8 | 1 | | |
| 5 | 2 | | □ | ■ | □ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | 0.5 | 1 |
| 6 | 27 | | ■ | ■ | □ | ■ | ■ | □ | □ | □ | ■ | □ | □ | ■ | ■ | ■ | □ | ■ | | | 0.5 | 1 |
| 7 | 8 | | ■ | ■ | □ | ■ | ■ | ■ | □ | □ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | 4.0 | 5 | 4 | 8 |
| 8 | 10 | | ■ | ■ | □ | ■ | ■ | ■ | □ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | | | 3.0 | 6 |
| 9 | 12 | | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | 1.6 | 2 | 1 | 2 |
| 10 | 19 | | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | 1 | 2 |
| 11 | 18 | | □ | ■ | ■ | □ | □ | ■ | ■ | ■ | □ | □ | ■ | ■ | ■ | ■ | ■ | ■ | 3.2 | 4 | 1 | 2 |
| 12 | 58 | | □ | ■ | ■ | □ | □ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | 0.5 | 1 |
| 13 | 4 | | □ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 4.8 | 6 | 1 | 2 |
| 14 | 31 | | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | ■ | ■ | ■ | ■ | ■ | ■ | | | 0.5 | 1 |
| 15 | 9 | | ■ | ■ | ■ | ■ | □ | □ | ■ | □ | □ | □ | □ | ■ | ■ | ■ | ■ | ■ | | | 1 | 2 |
| 16 | 3 | | ■ | ■ | ■ | ■ | □ | ■ | □ | □ | □ | □ | □ | ■ | ■ | ■ | ■ | ■ | 0.8 | 1 | 1 | 2 |
| 17 | 5 | | ■ | ■ | ■ | ■ | □ | ■ | □ | □ | □ | □ | ■ | ■ | ■ | ■ | ■ | ■ | 0.8 | 1 | | |
| 18 | 13 | | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | □ | □ | ■ | ■ | ■ | ■ | ■ | ■ | 8.0 | 10 | 4 | 8 |
| 19 | 11 | | ■ | ■ | ■ | ■ | □ | ■ | □ | □ | □ | ■ | □ | ■ | ■ | ■ | ■ | ■ | 2.4 | 3 | | |
| 20 | 7 | | ■ | ■ | ■ | ■ | □ | ■ | ■ | □ | □ | ■ | □ | ■ | ■ | ■ | ■ | ■ | | | 1 | 2 |
| 21 | 382 | | ■ | ■ | ■ | ■ | □ | ■ | ■ | □ | □ | ■ | □ | ■ | ■ | ■ | ■ | ■ | 1.6 | 2 | | |
| 22 | 6 | | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 2.4 | 3 | | |
| 23 | 144 | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | □ | ■ | ■ | ■ | ■ | ■ | ■ | | | 0.5 | 1 |
| 24 | 190 | | ■ | ■ | ■ | ■ | ■ | □ | □ | □ | ■ | □ | □ | ■ | ■ | ■ | ■ | ■ | 8.8 | 11 | 15 | 30 |
| 25 | 81 | | ■ | ■ | ■ | ■ | ■ | □ | □ | □ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | 0.8 | 1 | | |
| 26 | 297 | | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | 0.8 | 1 | 1.5 | 3 |
| 27 | 180 | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | 5.6 | 7 | 3.5 | 7 |
| 28 | 317 | | ■ | ■ | ■ | ■ | ■ | ■ | □ | □ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | 9.6 | 12 | 11 | 22 |
| 29 | 71 | | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | | | 5.5 | 11 |
| 30 | 113 | | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | 2.5 | 5 |
| 31 | 90 | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | | | 0.5 | 1 |
| 32 | 73 | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | 4 | 5 | 5.5 | 11 |
| 33 | 308 | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 8.8 | 11 | 12.5 | 25 |
| 34 | 69 | | ■ | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | ■ | ■ | ■ | ■ | ■ | 1.6 | 2 | | |

The presence and absence of a KIR gene are indicated by a shaded and white box, respectively. KIR ID assigned by the Allele Frequency Net Database

only present in one individual and were confirmed with sets of primers designed by Vilches et al. [13] and Ashouri et al. [14].

## Discussion

KIR–HLA interactions determine the diversity of NK cell function; so that different combinations of KIR–HLA may affect susceptibility or resistance to some diseases [16]. According to our results, *KIR2DL2* and its activating

counterpart; *KIR2DS2* are less frequent in UBC patients compared with controls [17].

KIR2DL2 and KIR2DL3 are inhibitory receptors which interact with HLAC1 and induce the inhibitory signals that dampen NK cell function; the KIR2DL2 ligation is much stronger because of polymorphisms at two positions in ligand-binding site [18]. In accordance with our result, the protective role of KIR2DL2 was demonstrated in patients with breast cancer, kidney disease and patients infected by HIV-1 [19] while it's predisposing role was reported in leukemia [20].

KIR2DS2 which can bind HLAC1 with lower affinity than KIR2DL2, is associated with inflammatory condition, increased amount of IFN-γ secretion in patients infected by HIV-1 [19] and some autoimmune disorders [21] such as type 1 diabetes, rheumatoid arthritis, vasculitis and scleroderma [22]. Similar to our data, the frequency of KIR2DS2 was lower in patients with HCV infection while in contrast with our result, its presence has been shown a protective role against colorectal cancer [23].

Decrease in the presence of *KIR2DS2* and *KIR2DL2* would imply that the most of UBC patients are probably have A haplotype than B. We observed a non-significant increase in AA genotypes in these patients than controls, while a lower frequency of AA genotype was reported in Iranians with head and neck squamous cell carcinomas than controls [24].

It is presumable that the *KIR2DS2* and *KIR2DL2* absence, result in more inhibition of NK cells in UBC patients. Since KIR2DL2 are in strong linkage disequilibrium with KIR2DS2, lower frequencies of the carriers of both genes in UBC patients compared to controls remains to be studied in further studies as well as along with other candidate risk related genes in bladder cancer.

Considering the lower affinity of KIR2DL2 than KIR2DL3 for HLA-C1, the presence of KIR2DL3 in the absence of KIR2DL2, due to decrease in the inhibitory signals and as a result, the chronic activation in NK cell and induction of inflammation in tumor microenvironment [16] which is linked to the development and progression of many cancers [25, 26]. Furthermore, lack of *KIR2DS2* may be decrease the NK cell's activity and possibly hampers the immunosurveillance. Momot et al. introduced some rare genotypes which had *KIR2DS2* but lacking *KIR2DL2* and were associated to susceptibility to scleroderma. It has been shown that *KIR2DS2* in the absence of HLAC1 can favor to NK cell activation [27].

Furthermore, our results show that CxT4 genotype which contains more activating receptors is significantly more frequent in UBC patients which is consistent with our previous research findings that the frequency of CxT4 genotype carriers was higher in patients with head and neck, laryngeal and oral cavity squamous cell carcinomas compared with healthy controls [24]. It is assumed that the absence of inhibitory *KIR2DL2* in CxT4 cluster along with unknown tumoral antigenic stimuli as well as chronic inflammatory context of infectious agent associated cancers such as bladder cancer may contribute to sustainable NK cell chronic activation that increases the likelihood of development of cancer through local inflammation. Study of *KIR* genes diversity in thyroid and cervical cancer patients indicates that the frequency of activating *KIR* genes is higher in patients compared to controls, a finding which is consistent with the current study and interpretation [28, 29].

Future studies of *KIR*/*HLA* genes diversity in patients with bladder cancer as well as, functional studies of NK cells lacking KIR2DL2 and KIR2DS2 would be valuable strategy to find out the effect of their simultaneous absence on the threshold of NK cell activation and NK cell meddling in cancer immunity.

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## Compliance with ethical standards

**Conflict of interest** The authors have declared no conflicting interest.

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