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# KIR-HLA gene diversities and susceptibility to lung cancer

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Killer-cell immunoglobulin-like receptors (KIR) are essential for acquiring natural killer (NK) cell effector function, which is modulated by a balance between the net input of signals derived from inhibitory and activating receptors through engagement by human leukocyte antigen (HLA) class I ligands. KIR and HLA loci are polygenic and polymorphic and exhibit substantial variation between individuals and populations. We attempted to investigate the contribution of *KIR* complex and *HLA class I* ligands to the genetic predisposition to lung cancer in the native population of southern Iran. We genotyped 16 *KIR* genes for a total of 232 patients with lung cancer and 448 healthy controls (HC), among which 85 patients and 178 HCs were taken into account for evaluating combined *KIR-HLA* associations. *KIR2DL2* and *2DS2* were increased significantly in patients than in controls, individually (OR 1.63, and OR 1.42, respectively) and in combination with *HLA-C1* ligands (OR 1.99, and OR 1.93, respectively). *KIR3DS1* (OR 0.67) and *2DS1* (OR 0.69) were more likely presented in controls in the absence of their relative ligands. The incidence of CxTx subset was increased in lung cancer patients (OR 1.83), and disease risk strikingly increased by more than fivefold among genotype ID19 carriers (a CxTx genotype that carries *2DL2* in the absence of *2DS2*, OR 5.92). We found that genotypes with *iKIRs* > *aKIRs* (OR 1.67) were more frequently presented in lung cancer patients. Additionally, patients with lung cancer were more likely to carry the combination of CxTx/*2DS2* compared to controls (OR 2.04), and *iKIRs* > *aKIRs* genotypes in the presence of *2DL2* (OR 2.05) increased the likelihood of lung cancer development. Here we report new susceptibility factors and the contribution of *KIR* and *HLA-I* encoding genes to lung cancer risk, highlighting an array of genetic effects and disease setting which regulates NK cell responsiveness. Our results suggest that inherited *KIR* genes and *HLA-I* ligands specifying the educational state of NK cells can modify lung cancer risk.

Lung cancer, the most common cause of cancer-related mortality worldwide is generally classified into main histological subtypes, including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC)<sup>1,2</sup>. Lung mucosa is constantly exposed to inhaled environmental pollution, dust, smoke, and pathogens; hence a dynamic network of tissue-resident immune cells continually keeps monitoring the lung to maintain tissue homeostasis<sup>3</sup>. The immune system also has a vital role in cancer initiation and progression. Natural killer cells (NK cells) are part of innate immune cells that serve as a first-line of defense with the capacity to eliminate virally infected cells and neoplastic transformations without prior sensitization<sup>4</sup>. These cytotoxic cells mediate antitumor responses by exploiting death receptors, releasing pro-inflammatory cytokines and perforin/granzyme granules exocytosis<sup>5</sup>.

Interestingly, over 10% of resident lymphocytes in the lung are NK cells<sup>6</sup>. The association of NK cell dysfunction with tumor progression was revealed in KRAS-driven lung cancer, in which NK cells protect tumors at the early stage but cannot prevent tumor progression<sup>7</sup>. Also, it is shown that resident NK cells have a pivotal role in resistance to experimental lung metastasis by producing IFN- $\gamma$ <sup>8</sup>. Although the infiltration of NK cells was indicated as a favorable prognostic factor in lung cancer<sup>9,10</sup>, the functional reactivity of intratumoral NK cells is crucial in addition to the degree of NK cell infiltration. It has been demonstrated that NK cell dysfunction at the late stage is caused by the suppressive tumor microenvironment (TME)<sup>7</sup>. NK cells isolated from NSCLC patients, which depict different expression patterns, have impaired interferon- $\gamma$  (IFN- $\gamma$ ) production capability and lower cytotoxicity than non-tumoral NK cells<sup>11–13</sup>.

The activity of NK cells is modulated by a balance between the net input of signals derived from an array of inhibitory and activating receptors<sup>14</sup>. Among these germ-line encoded receptors, killer-cell immunoglobulin-like

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Characteristics	Lung cancer patients (n = 232)	Healthy controls (n = 448)
Mean age $\pm$ SD	64.4 $\pm$ 11.1	58.13 $\pm$ 12.66
<b>Gender</b>		
Female	34 (14.7%)	150 (33.5%)
Male	198 (85.3%)	298 (66.5%)
<b>Smoking status</b>		
Smoker	51 (21.98%)	64 (14.3%)
Non-smoker	0 (0.0%)	3 (0.7%)
Unknown	181 (78.02%)	381 (85.0%)
<b>Lung cancer subtypes</b>		
SCLC	53 (22.8%)	
NSCLC	179 (77.2%)	
Squamous cell carcinoma	144 (62.1%)	
Adenocarcinoma	35 (15.1%)	

**Table 1.** Characteristics of the study population.

receptors (KIR) are essential for acquiring NK cell effector function<sup>15,16</sup>. KIRs are encoded by a cluster of polymorphic and homologous genes located at chromosomal region 19q13.4<sup>17</sup>. KIR region comprises activating (*2DS1-5*, *3DS1*) and inhibitory (*2DL1-3*, *2DL5A/B*, *3DL1*) genes, divided by framework KIRs into centromeric (*3DL3* to *3DP1*) and telomeric (*2DL4* to *3DL2*) intervals<sup>18</sup>. Although (5') centromeric and (3') telomeric regions contain various combinations of activating (*aKIR*) and inhibitory (*iKIR*) genes, certain adjacent KIRs tend to be strongly linked<sup>17,19</sup>. Considering strong linkage disequilibrium (LD) among *KIR* genes, it is difficult to isolate each *KIR* gene's effect on NK cell response<sup>20</sup>. Based on gene content within both intervals, the KIR locus segregates in distinct haplotypes, mainly known as A (fixed combination of genes, mostly *iKIRs*) and B (variable number of *iKIRs/aKIRs*)<sup>17,21</sup>. The KIR gene cluster displays tremendous variation due to gene-content diversity and haplotypic variety<sup>17</sup>. In addition to gene content diversity, allelic polymorphism extends KIR variations and accounts for differential expression levels of KIRs on the surface of NK cells<sup>22,23</sup>.

The classical HLA class I molecules (HLA-A, B, C) as major KIR ligands have been categorized into 4 types of KIR-binding epitopes (C1, C2, Bw4, A3/A11) according to amino acid sequences<sup>24</sup>. KIR2DL1 and KIR2DL2/3 mainly bind to a group of ligands encoded by HLA-C alleles that differed by Lys/Asn dimorphism at position 80 (HLA-C2 and HLA-C1, respectively)<sup>25</sup>. KIR3DL1 was found to interact with HLA-Bw4, and KIR3DL2 recognizes HLA-A3/A11<sup>26,27</sup>. The Ile/Thr dimorphism at position 80 defines the affinity of KIR binding to the Bw4 motif, in which Ile80 exhibits a greater affinity for 3DL1<sup>28</sup>. The growing understanding of activating KIR/HLA interactions indicates that activating KIR3DS1, 2DS1, and 2DS2 bind to the same HLA class I with lower affinity than their homologous inhibitory counterparts<sup>29-32</sup>. However, other KIR/HLA pair interactions haven't been distinctly demonstrated. It is shown that different *iKIRs* exhibit various binding affinities for HLA-I ligands. Interestingly the peptide presented by HLA subtypes seems to play a role in binding affinity alteration<sup>33</sup>. Signals derived from *iKIRs* interacting with self-HLA ligands set a threshold of activation for NK cells leading to a maturation process titled "education" or "licensing"<sup>34,35</sup>. Besides development and self-tolerance, education renders NK cells capacity to recognize diseased cells with downregulated or lacking HLA class I expression, referring to the "missing self" hypothesis<sup>34</sup>. Lacking a specific HLA-I ligand or any responding *iKIR*, yields hypo-responsive NK clones with high activation threshold<sup>30</sup>. It is particularly noteworthy that *KIR*-gene complex diversity influences surface expression, ligand specificities, ligand binding affinity, and subsequent signal transduction through KIR-HLA class I interaction<sup>36-38</sup>. Such an intense heterogeneity in *KIR* and *HLA* complex prominently affects NK cell responses and is associated with disease susceptibility<sup>39</sup>. Genome-wide association studies (GWAS) have identified 45 loci associated with lung cancer risk<sup>40</sup>. More specifically, SNPs from 5p15.33, 6p21.33, and 15q25.1 regions are strongly associated with lung cancer in Caucasians<sup>41</sup>. However, the composition of multiple highly-homogeneous gene content, intense polymorphism, strong linkage disequilibrium between multiple loci, and low/no coverage by GWAS reagents pose challenges to studying the polymorphism of KIR (19q13) and HLA (6p21) gene families by GWAS.

The present study aimed to investigate the contribution of *KIR* complex and *HLA-I* ligands to the genetic predisposition to lung cancer in the native population of Fars province, located in the southern part of Iran, and to disclose possible associations with the dysfunctional state of NK cells in the context of lung cancer (Table 1).

## Results

**Susceptibility/resistance influence of specific B haplotype-associated KIRs on lung cancer risk.** The distribution of 16 KIR genes were determined in patients and HCs (Table 2). As observed, framework genes (*3DL3*, *3DP1*, *2DL4*, *3DL2*) were presented in all subjects. Two adjacent B haplotype-associated genes *2DL2* (67.2% vs. 55.4%,  $p=0.003$ , OR 1.63, CI 1.17–2.26) and *2DS2* (62.9% vs. 54.6%,  $p=0.041$ , OR 1.42, CI 1.02–1.96) were significantly increased in patients in comparison with controls. Activating genes *2DS1* (50.2% vs. 41.4%,  $p=0.029$ , OR 0.69, CI 0.5–0.95) and *3DS1* (47.3% vs. 37.5%,  $p=0.015$ , OR 0.67, CI 0.48–0.92) were more frequently presented in controls than patients, conferring protection against the lung cancer. Table 2

KIR genes	Healthy controls n = 448 %F (N + /n)	Lung cancer			Comparisons					
		Lung cancer n = 232 %F (N + /n)	NSCLC n = 179 %F (N + /n)	SCLC n = 53 %F (N + /n)	Lung cancer versus HC		NSCLC versus HC		SCLC versus HC	
		<i>p</i> value	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value	OR (95% CI)			
<b>Group-A haplotype-associated KIR genes</b>										
<i>2DL1</i>	98.0 (439/448)	97.4 (226/232)	97.2 (174/179)	98.1 (52/53)						
<i>2DL3</i>	88.2 (395/448)	85.3 (198/232)	84.4 (151/179)	88.6 (47/53)						
<i>3DL1</i>	92.6 (415/448)	95.3 (221/232)	96.6 (173/179)	92.4 (49/53)						
<i>2DS4</i>	92.9 (416/448)	94.8 (220/232)	95.5 (171/179)	92.4 (49/53)						
<i>2DS4fl</i>	12.9 (31/242)	10.4 (18/174)	10.2 (14/137)	10.8 (4/37)						
<i>2DS4del</i>	73.5 (178/242)	70.6 (123/174)	70.8 (97/137)	70.3 (26/37)						
<i>2DS4fl,del</i>	13.6 (33/242)	19.0 (33/174)	19.0 (26/137)	18.9 (7/37)						
<b>Group-B haplotype-associated KIR genes</b>										
<i>2DL2</i>	55.4 (248/448)	67.2 (156/232)	65.9 (118/179)	71.6 (38/53)	0.003	1.63 (1.17–2.26)	0.016	1.56 (1.087–2.24)	0.027	2.04 (1.09–3.82)
<i>2DL5</i>	66.1 (296/448)	63.8 (148/232)	64.2 (115/179)	62.2 (33/53)						
<i>3DS1</i>	47.3 (212/448)	37.5 (87/232)	36.8 (66/179)	39.6 (21/53)	0.015	0.67 (0.48–0.92)	0.02	0.65 (0.43–0.93)		
<i>2DS1</i>	50.2 (225/448)	41.4 (96/232)	41.3 (74/179)	41.5 (22/53)	0.029	0.69 (0.5–0.95)				
<i>2DS2</i>	54.5 (244/448)	62.9 (146/232)	62.0 (111/179)	66.0 (35/53)	0.041	1.42 (1.02–1.96)				
<i>2DS3</i>	41.5 (186/448)	40.1 (93/232)	39.6 (71/179)	41.5 (22/53)						
<i>2DS5</i>	39.5 (177/448)	32.8 (76/232)	32.9 (59/179)	32.0 (17/53)						
<b>Framework genes/pseudogenes</b>										
<i>2DL4</i>	100 (448)	100 (232)	100 (179)	100 (53)						
<i>3DL2</i>	100 (448)	100 (232)	100 (179)	100 (53)						
<i>3DL3</i>	100 (448)	100 (232)	100 (179)	100 (53)						
<i>2DP1</i>	97.8 (438)	96.1 (223)	94.9 (170)	100 (53)						
<i>3DP1</i>	100 (448)	100 (232)	100 (179)	100 (53)						

**Table 2.** KIR gene frequencies among patients with lung cancer and healthy controls. *N*+ number of individuals positive for the gene, *n* number of individuals tested for the gene, *HC* healthy control, *OR* odds ratio, *CI* confidence interval.  $p < 0.05$ : statistically significant; based on two-tailed Fisher's exact test.

also shows the results with further assessment of *KIR* genes and their associations with lung cancer subtypes (NSCLC, SCLC).

**Risk-association of KIR-HLA combinations with lung cancer.** To evaluate the contribution of *KIR*-*HLA* combinations to lung cancer risk, we analyzed the distribution of *KIR* genes and cognate *HLA-I* ligands within a group of 85 patients along with 178 HCs (Table 3). Similar to the results with individual *KIR* genes, coexistence of *2DL2/CI* (55.3% vs. 38.3%,  $p = 0.026$ , OR 1.99, CI 1.11–3.56) and *2DS2/CI* (50.8% vs. 34.7%,  $p = 0.036$ , OR 1.93, CI 1.08–3.46) were found to occur more frequently in lung cancer patients than controls. A significantly less frequent carriage of *3DL1-Bw4* combination was detected in patients with lung cancer than HCs (44.2% vs. 62%,  $p = 0.014$ , OR 0.48, CI 0.25–0.91). However, no significant differences were observed between the two groups for the prevalence of *2DS1* and *3DS1* genes combined with respective *HLA-C2* and *HLA-Bw4* ligands, suggesting that particular educational states of NK cells may alter NK cell functionality in the lung cancer setting.

When *HLA-I* ligands were analyzed separately, *HLA-Bw4 (Ile80)* was less prevalent (3.8% vs. 16.3%,  $p = 0.018$ , OR 0.2, CI 0.47–0.9) in lung cancer patients than in controls (Table 3). This points to the possibility that the difference in the distribution of *3DL1-Bw4* combination could be driven by *Bw4 (Ile80)*. The results of comparing the frequency of remaining *HLA-I* genes didn't reach the level of statistical significance.

Subgroup analysis wasn't accomplished regarding the association of *KIR*-*HLA* combinations and *HLA-I* ligands with different lung cancer subtypes owing to the inadequate sample size included in the HLA typing method.

**Susceptibility/resistance influence of specific Bx genotype-associated gene clusters on lung cancer risk.** *KIR* genotype profiles of 232 lung cancer patients and 448 HCs are listed in Table 4. A set of 65 genotypes differentiated by *KIR* gene content were detected in a total of 680 study participants from southern Iran. Thirty-three genotypes occurred in both patients and controls, 13 genotypes occurred only in patients, and 19 genotypes occurred only in controls. Genotype ID5 (12.1% vs. 7.4%,  $p = 0.048$ , OR 1.73, CI 1.015–2.93) was significantly more frequent in patients in comparison with controls. Strikingly, the genotype ID19, which is a rare CxTx genotype carrying *2DL2* in the absence of *2DS2* was associated with a more than fivefold increase in lung cancer risk (2.4% vs. 0.4%,  $p = 0.021$ , OR 5.92, CI 1.18–29.58) (Fig. 1).

The distribution of main genotypes AA and Bx were comparable among the two groups. Significant differences in the frequencies of Bx genotype subsets categorized based on C4 and T4 gene clusters were observed, whereby

KIR/HLA	Healthy controls	Lung cancer	Lung cancer versus HC	
	n = 448	n = 232	p value	OR (95% CI)
	%F (N + /n)	F% (N + /n)		
<b>KIR-binding motif</b>				
HLA-C1	74.2 (124/167)	81.5 (53/65)		
HLA-C2	73.6 (123/167)	72.3 (47/65)		
HLA-Bw4	62.1 (95/153)	48.1 (25/52)		
Bw4T80	45.7 (70/153)	44.2 (23/52)		
Bw4I80	16.3 (25/153)	3.8 (2/52)	0.018	0.2 (0.047–0.9)
HLA-A3/A11	39.2 (31/79)	38.1 (24/63)		
HLA-A23/24/25/32	49.3 (39/79)	44.4 (28/63)		
<b>KIR-HLA combination</b>				
3DL2 + A3/A11	39.2 (31/79)	38.1 (24/63)		
2DL1 + C2	71.8 (120/167)	72.3 (47/65)		
2DL3 + C1	68.8 (115/167)	69.2 (45/65)		
2DL2 + C1	38.3 (64/167)	55.3 (36/65)	0.026	1.99 (1.11–3.56)
3DL1 + Bw4	62.0 (95/153)	44.2 (23/52)	0.014	0.48 (0.25–0.91)
2DS1 + C2	26.6 (45/169)	27.7 (18/65)		
2DS2 + C1	34.7 (58/167)	50.8 (33/65)	0.036	1.93 (1.08–3.46)
3DS1 + Bw4	20.2 (31/153)	15.3 (8/52)		

**Table 3.** Frequency of KIR-HLA combinations, and HLA class-I ligands among patients with lung cancer and healthy controls. N+ number of individuals positive for the gene, n number of individuals tested for the gene, HC healthy control, OR odds ratio, CI confidence interval.  $p < 0.05$ : statistically significant; based on two-tailed Fisher's exact test.

KIR	Healthy controls	Lung cancer				Comparisons				
		Lung cancer	NSCLC	SCLC	Lung cancer versus HC	NSCLC versus HC		SCLC versus HC		
	n = 448	n = 232	n = 179	n = 53	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)
	%F (N+)	%F (N+)	%F (N+)	%F (N+)						
<b>KIR genotypes</b>										
AA	25.7 (115)	19.4 (45)	19.5 (35)	16.9 (9)						
Bx	74.3 (333)	80.6 (187)	80.4 (144)	83.1 (44)						
CxT4	22.7 (102)	17.6 (41)	18.4 (33)	15.0 (8)						
C4Tx	23.4 (105)	28.4 (66)	29.6 (53)	24.5 (13)						
C4T4	12.7 (57)	9.5 (22)	8.37 (15)	13.2 (7)						
CxTx	15.4 (69)	25.0 (58)	24.0 (43)	30.2 (16)	0.0035	1.83 (1.23–2.71)	0.015	1.74 (1.13–2.66)	0.011	2.37 (1.25–4.5)
C4 Linkage groups	36.2 (162)	37.9 (88)	37.9 (68)	37.7 (20)						
T4 Linkage groups	35.4 (159)	27.1 (63)	26.8 (48)	28.3 (15)	0.03	0.67 (0.47–0.97)	0.0039	0.66 (0.45–0.97)		

**Table 4.** KIR genotype frequencies among patients with lung cancer and healthy controls. N+ number of individuals positive for the gene, n number of individuals tested for the gene, HC healthy control, OR odds ratio, CI confidence interval.  $p < 0.05$ : statistically significant; based on two-tailed Fisher's exact test.

T4 gene cluster (35.4% vs. 27.1%,  $p = 0.03$ , OR 0.67, CI 0.47–0.97) was found to be associated with reduced risk of lung cancer in our study population. In contrast, we noticed significantly more frequent CxTx subset (25% vs. 15.4%,  $p = 0.0035$ , OR 1.83, CI 1.23–2.71) in patients indicating an association between this subset and increased risk of lung cancer (Table 4). KIR genotype frequencies and their statistical associations with lung cancer subtypes (NSCLC, SCLC) are summarized in Table 4.

**Risk-association of co-existing susceptibility factors with lung cancer.** To explore further the possible associations of carrying gene contents varied in the number of inhibitory and activating KIR genes on susceptibility to lung cancer, we assessed comparisons with regard to different numbers of *iKIRs* and *aKIRs* (Table 5). Carriage of genotypes with *iKIRs* > 4 was more likely presented in patients (75% vs. 67%,  $p = 0.035$ ,  $P_c = 0.14$ , OR 1.48, CI 1.036–2.11), on the contrary genotypes with *aKIRs* > 4 were more frequently found in HCs (50% vs. 40.1%,  $p = 0.015$ ,  $P_c = 0.06$ , OR 0.66, CI 0.48–0.92). Lung cancer patients were more likely to carry genotypes with *iKIRs* > *aKIRs* (64.7% vs. 52.2%,  $p = 0.002$ ,  $P_c = 0.008$ , OR 1.67, CI 1.2–2.32), and this difference remained significant after being corrected for multiple comparisons, suggesting the strong association of genotypes with *iKIRs* > *aKIRs* with susceptibility to lung cancer.

Genotypes			Group-A associated <i>KIR</i> genes				Group-B associated <i>KIR</i> genes						Framework/Pseudogenes			Healthy controls		Lung cancer					
genotype ID	Group	Bx-Subset	2DL1	2DL3	3DL1	2DS4	2DS2	2DL2	2DS3	2DL5	3DS1	2DS5	2DS1	2DP1	3DP1	2DL4	3DL2	3DL3	%F	(N+)	%F	(N+)	
			1	AA																		25.2	(113)
203																			0.4	(2)	0.4	(1)	
5*	Bx	C4Tx																	7.4	(33)	12.1	(28)	
7																				5.1	(23)	5.6	(13)
71																				3.1	(14)	4.7	(11)
11																				1.6	(7)	1.7	(4)
90																				1.8	(8)	0.4	(1)
13																				1.1	(5)	1.3	(3)
91																				0.7	(3)	0.4	(1)
382																				0.7	(3)		
159																				0.4	(2)		
113																				0.4	(2)		
22																			0.2	(1)			
190																			0.2	(1)			
239																			0.2	(1)			
25																					0.9	(2)	
94																			0.2	(1)	0.9	(2)	
112																			0.2	(1)	0.4	(1)	
6		C4T4																	8.3	(37)	5.6	(13)	
73																			2.5	(11)	1.3	(3)	
70																			1.3	(6)	0.9	(2)	
81																			0.7	(3)	1.3	(3)	
93																					0.4	(1)	
2	CxT4																	10.5	(47)	9.1	(21)		
3																		4.9	(22)	2.2	(5)		
69																		2.0	(9)	0.4	(1)		
28																		1.6	(7)	0.9	(2)		
18																		1.1	(5)	1.3	(3)		
88																		0.7	(3)				
68																		0.4	(2)	1.3	(3)		
75																		0.4	(2)	0.4	(1)		
12																		0.2	(1)	0.4	(1)		
144																		0.2	(1)				
166																		0.2	(1)				
359																		0.2	(1)				
76																		0.2	(1)				
79																				0.4	(1)		
429																				0.9	(2)		
595																			0.4	(1)			
4	CxTx																	6.5	(29)	10.8	(25)		
31																		1.6	(7)	0.4	(1)		
8																		1.6	(7)				
9																		1.1	(5)	1.7	(4)		
19**																		0.4	(2)	2.6	(6)		
381																				0.4	(1)		
117																		0.4	(2)				
104																		0.4	(2)				
200																		0.4	(2)	0.4	(1)		
106																		0.4	(2)	0.9	(2)		
337																		0.4	(2)	0.4	(1)		
89																		0.2	(1)	0.9	(2)		
64																		0.2	(1)				
30																		0.2	(1)				
23																		0.2	(1)				
36																		0.2	(1)				
240																		0.2	(1)				
29																				1.7	(4)		
72																		0.2	(1)	1.3	(3)		
92																				0.9	(2)		
27																			0.4	(1)			
336																			0.4	(1)			
21																	0.4	(2)	0.4	(1)			
17																			0.4	(1)			
293																			0.4	(1)			
188																			0.4	(1)			
Total genotypes found in each population																			52		46		

**Figure 1.** KIR gene content diversity among patients with lung cancer and healthy controls. N+ : number of individuals positive for the gene; n: number of individuals tested for the gene; Gene content of 65 KIR genotypes are displayed by presence/shaded boxes or absence/white boxes of 16 KIR genes. Distribution of genotypes with ID5, and ID19 highlighted by dark boxes were found to be significantly different between lung cancer patients and controls. \**p* = 0.048, OR 1.73, 95% CI: (1.015–2.93); \*\**p* = 0.021, OR 5.92, 95% CI: (1.18– 29.58).

KIR combination	Healthy controls	Lung cancer	Lung cancer versus HC		
	n = 448	n = 232	p value	P <sub>c</sub>	OR (95% CI)
%F (N + /n)	F% (N + /n)				
<b>KIR genotypes</b>					
<i>iKIR</i> > <i>aKIR</i>	52.2 (234/448)	64.7 (150/232)	0.002	0.008*	1.67 (1.2–2.32)
<i>aKIR</i> > <i>iKIR</i>	19.4 (87/448)	13.4 (31/232)			
<i>iKIR</i> > 4	67.0 (300/448)	75.0 (174/232)	0.035	0.14*	1.48 (1.036–2.11)
<i>aKIR</i> > 4	50.0 (224/448)	40.1 (93/232)	0.015	0.06*	0.66 (0.48–0.92)
<b>KIR combined genotypes</b>					
CxTx/2DS2	11.1 (50/448)	20.2 (47/232)	0.0074	0.066**	1.84 (1.18–2.88)
CxTx/2DL2	12.3 (55/448)	23.7 (55/232)	0.0012	0.011**	2.04 (1.34–3.13)
<b><i>iKIR</i> &gt; <i>aKIR</i> carriers subgroup</b>					
2DS2 presence	45.2 (105/232)	59.3 (89/150)	0.0087	0.078**	1.76 (1.16–2.67)
2DL2 presence	49.1 (114/232)	66.6 (100/150)	0.0008	0.0072**	2.05 (1.35–3.17)

**Table 5.** Carrier frequency of various susceptibility-related factor combinations among patients with lung cancer and healthy controls. N+ number of individuals positive for the gene, n number of individuals tested for the gene, HC healthy control, OR odds ratio, CI confidence interval.  $p < 0.05$ : statistically significant; based on two-tailed Fisher's exact test; P<sub>c</sub>: corrected p values, P<sub>c</sub>\*: correction factor = 4, P<sub>c</sub>\*\* : correction factor = 9.

We next performed a comparative analysis to explore whether simultaneous inheritance of disease risk-related factors influences disease susceptibility (Table 5). We found that patients with lung cancer were more likely to carry the combination of CxTx/2DS2 compared to controls (20.2% vs. 11.1%,  $p = 0.0074$ ,  $P_c = 0.066$ , OR 1.84, CI 1.18–2.88). The likelihood of carrying CxTx/2DL2 combination was also significantly higher in patients (23.7% vs. 12.3%,  $p = 0.0012$ ,  $P_c = 0.011$ , OR 2.04, CI 1.34–3.13). Likewise, disease susceptibility was conferred by the presence of 2DL2 within individuals carrying *iKIRs* > *aKIRs* (66.6% vs. 49.1%,  $p = 0.0008$ ,  $P_c = 0.0072$ , OR 2.05, CI 1.35–3.17), this association was weakened in the presence of 2DS2 (59.3% vs. 45.2%,  $p = 0.0087$ ,  $P_c = 0.078$ , OR 1.76, CI 1.16–2.67).

## Discussion

In the present study, we assessed the contribution of *KIR* gene content and their corresponding *HLA-I* ligands to lung cancer development in the ethnically homogeneous population of southern Iran. Although previous studies have examined *KIRs* at genetic, transcriptional, and expression levels in lung cancer, to our knowledge, this is the first report that demonstrates individual *KIR* genes and certain genotypes seem to be associated with susceptibility to lung cancer. Given our sizeable dataset, suggestive interactions between *KIR*-*HLA* class I ligands can influence the dynamics of NK cell responses in the lung cancer setting.

Despite existing research addressing the *KIR*-*HLA* pair's role in lung cancer, their findings are less consistent. Most recently, in the Chinese Han population, studies conducted by Li et al.<sup>42</sup> and Yu et al.<sup>43</sup>, found no association between *KIRs* and *KIR*-*HLA* combinations with metastatic NSCLC (mNSCLC) and adenocarcinoma, respectively. Consistent with these findings, Wisniewski et al. couldn't find a significant difference between *KIR* genes or combinations of *KIR*-*HLA* in 269 Polish Caucasians with NSCLC compared with 690 HCs<sup>44</sup>. However, Wisniewski et al. reported carriers of homozygous *HLA-C1* and *C2* were more frequent in NSCLC patients, which was not detected in our study<sup>44</sup>. Furthermore, Al Omar et al. observed significantly increased *2DL1/C2* and decreased *2DL3/C1* in NSCLC patients from England and Northern Ireland<sup>45</sup>. Decreased frequency of *Ile80* allele in NSCLC patients positive for *3DL1/Bw4* and decreased *Thr80* allele in SCLC patients positive for *3DS1/Bw4*, which is observed by Al Omar et al.<sup>45</sup>, Conforms to our findings of less frequent *3DS1*, *3DL1/Bw4* in lung cancer patients. In part, these inconsistent results may be elucidated by the small sample size, heterogeneity of the target population (study population), and divergent distribution of *KIRs* and *HLAs* in different ethnic groups. Importantly, cross-talk of NK cells and the unique microenvironment of each lung cancer subtype<sup>46</sup> could be responsible for behavioral differences in NK cells, assessing distinct histologic subtypes including (mNSCLC, NSCLC, and SCLC) can presumably lead to conflicting results observed in mentioned studies.

Although we didn't examine survival rate and response to treatment in lung cancer patients, previous studies obtained interesting results. Yu et al. noted that chemotherapy-treated mNSCLC patients with *KIR2DS4del* and *HLA-Bw4* (*Thr80*) gene expression at the mRNA level exhibited poor overall survival (OS)<sup>43</sup>. Wisniewski et al. reported the striking association of *2DL2/2DS2/C1* combination with more prolonged survival and better response to therapy in Polish patients<sup>44</sup>, which is discordant with the predisposing effect of *2DL2/C1*, *2DS2/C2* on lung cancer risk observed in our study. Given the cancer setting, it is crucial to consider the impact of chemotherapy agents on the sensitization of tumor cells to NK cell activity. As it has been shown that stress signals induced by chemotherapy and other treatment modalities can elevate the expression of NK cell-activating ligands<sup>47–49</sup> or downregulate inhibitory ligands<sup>50,51</sup>, the transient deleterious effect of chemotherapeutic agents on NK cells has also been observed<sup>52,53</sup>.

Our findings primarily determined an association between the carriage of *KIR2DL2* and its activating counterpart *2DS2* with an increased risk of lung cancer. We identified similar results when further analyzing *2DL2*

and *2DS2* in the presence of their corresponding *HLA-C1* allele. Consistent with our results, *2DL2* has been demonstrated to confer susceptibility to endometriosis<sup>54</sup>, leukemia<sup>55</sup>, and could be predisposing to lymph node metastasis (LNM) in HNSCC as well<sup>56</sup>. The carrier frequency of *2DL2/C1* in malignant melanoma patients with the advanced stage was significantly higher compared with lower-stage patients<sup>57</sup>. Similar results were reported by Naumova et al. showing the association of *2DL2/C1* with malignant melanoma<sup>58</sup>. Additionally, *2DL2* and *2DS2* have been shown to confer a predisposition to lymphatic invasion in ER+ and PR+ breast cancer cases<sup>59</sup>.

According to the “licensing” model, NK cell education via inhibitory receptors integrating with cognate HLA-I ligands endows NK cells with full effector functions and self-tolerance<sup>34</sup>, while NK cell licensing by *iKIRs* translates into effective sensing of missing HLA I targets, “missing-self” hypothesis, *aKIR* mediated licensing in the presence of its HLA I ligand induces hypo-responsiveness and renders NK cells impaired responsiveness<sup>60</sup>. Analysis of infiltration pattern and immune cell localization in NSCLC patients revealed that HLA-I negative tumors are predominantly TIL-free and encapsulated by stromal tissue, which consists of a dense structure of FAP+ fibroblasts<sup>61</sup>. In addition, reduced TIL infiltration, bigger tumor size, and lymphatic spread have been observed among HLA-I/PD-L1+ tumors<sup>62</sup>. To this extent, our finding implies that NK cells of *2DL2/C1* carriers are incapable of mounting an efficient response against lung cancer tumors sustaining HLA-I expression due to a defect in “missing-self” recognition. Stromal tissue surrounding tumor lesions, which restrains TILs, including NK cells<sup>61</sup>, could hypothetically represent another immune escape mechanism to avoid NK cell attack in lung cancer with total loss or downregulated HLA-I. It is shown that *KIRs* recognize altered peptides presented by cognate HLA-I ligands<sup>63</sup>, indicating that alterations in peptide repertoire mostly occurring in the process of tumorigenesis could be detected by *KIRs*<sup>64</sup>. As a result, stimulation of *aKIRs* with neoantigens and tumor-inducible ligands expressed on lung cancer cells may prompt cytokine release instead of cytolytic function. Supporting examples would be recent studies in which  $\beta_2$ -microglobulin-independent ligand has been suggested to be recognized by *2DS2*<sup>65</sup> and *2DS4* interacting with melanoma-derived non-class I MHC proteins<sup>66</sup>. The *2DS2*-mediated education in the presence of *C1* ligand could raise activation threshold and cause hypo-responsiveness in carriers of *2DS2/C1* combination, it also remains possible that upregulated “induced self” ligands mentioned above are unable to overcome such hypo-responsiveness. Due to the tight LD between *2DS2/2DL2*, the co-carriage of this combination may exacerbate the detrimental impact attributed to individual genes. More investigation is needed to distinguish the predisposing effect of these two genes on lung cancer.

Moreover, the frequency of *KIR2DS1* and *3DS1* in the HC group was higher than in patients. We couldn't detect a significant association of *2DS1/C2*, *3DS1/Bw4* combinations with lung cancer, though the *HLA-Bw4 (Ile80)* allele and *KIR3DL1/Bw4* were strongly associated with protection against lung cancer. NK cells expressing *2DS1* exhibit an anergy state in individuals carrying *HLA-C2/C2*, but not in *HLA-C1/Cx* carriers<sup>67</sup>. Likewise, *Bw4 (Ile80)* could be recognized by *3DS1* positive NK cells derived from donors lacking *Bw4 (Ile80)*, in contrast with NK cells from donors positive for *Bw4 (Ile80)*<sup>68</sup>. Referring to these studies, it can be suggested that NK cells generated from those *3DS1* and *2DS1* carriers lacking putative *HLA-Bw4* and *HLA-C2* ligands have lower activation threshold and might recognize different ligands associated with lung tumor transformation. Therefore, carrying *3DS1* and *2DS1* in the absence of cognate HLA class I ligands could confer better protection against lung cancer. Supporting this explanation, HLA-F open conformers (OCs) have been reported as high-affinity ligands for *3DS1*<sup>69</sup>, and Kiani et al. have shown that *3DS1* positive NK cells could be activated upon ligation with HLA-F in which stimulation with HLA-F results in increased antiviral function in NK cells<sup>70</sup>. Interestingly a highly expressed level of HLA-F has been detected in lung cancer<sup>71</sup>, suggesting that HLA-F might be a factor in the association of *3DS1/2DS1* with protection in lung cancer. Furthermore *KIRs* exhibit various degrees of peptide selectivity, implying that *KIRs* are sensitive to altered peptides, and this sensory mechanism is more sensitive than “missing self-detection” of lowered HLA-I expression on target cells<sup>72</sup>. Regarding *iKIRs*, several studies have reported changing peptide repertoire in tumor cells function as peptide antagonism, which downmodulates NK cell inhibition by reducing inhibitory ligands on tumor cells, caused by low-affinity interaction of *KIR-HLA*<sup>73,74</sup>. The protective effect conferred by *3DL1/Bw4* could result from *3DL1* sensitivity to subtle alterations in presented peptides leading to a reduction in inhibitory signals and triggering cytotoxicity in the absence of HLA downregulation.

Estimating the immunological genetic profile could preferably characterize the pathogenesis of lung cancer. Our findings with more frequent T4 gene cluster carriers in HCs, which is likely imparted by the presence of *3DS1/2DS1* combination in T4 gene cluster, are in contravention of CxT4 predisposing role in head and neck squamous cell carcinoma and colorectal adenocarcinoma reported in our previous studies of the same population<sup>75,76</sup>. The association of CxTx with lung cancer risk is in line with susceptibility to meningioma reported in CxTx carriers<sup>77</sup>. The results of comparing the different number of *iKIR* and *aKIR* genes, in addition to signifying the strong positive association with lung cancer risk in individuals carrying more inhibitory genes; displayed the influence of activating genes on protection against disease regardless of not being significant after the *p* value correction.

As we emphasized the tight LD between *2DL2* and *2DS2*, it is difficult to dissect the contribution of individual *2DL2* and *2DS2* genes in susceptibility to the disease. Hence, assessing rare genotypes lacking either *2DL2* or *2DS2* is highly informative. Accordingly, we noticed a striking association of the genotype ID19, a rare CxTx genotype carrying *2DL2* in the absence of *2DS2*, with increased lung cancer risk by more than fivefold, suggesting a predominant detrimental impact of *2DL2* over *2DS2*. Regarding our analysis of co-existing susceptibility factors with lung cancer, simultaneous inheritance of CxTx/*2DL2* was shown to predispose carriers to lung cancer. Although, the simultaneous presence of CxTx/*2DS2* did not meet the significant level after *p* value correction and seems to confer a slightly lower risk of lung cancer compared to CxTx/*2DL2*. Superior adverse effect was noticeably related to individuals with *iKIR* > *aKIR* in the presence of *2DL2* rather than *2DS2*. Our findings are strengthened by the report denoting the correlation of higher expression in inhibitory *KIRs* with poor prognosis in lung cancer patients. A higher proportion of NK cells expressing inhibitory *KIRs* was noticed in NSCLC

patients in which lower cytotoxicity and reduced IFN- $\gamma$  production were also shown<sup>78</sup>. Consistently, separate consideration of inhibitory and activating counterparts demonstrated the susceptibility of *2DL1*<sup>+</sup>/*SI*<sup>-</sup> genotype carriers to cutaneous melanoma and the formation of sentinel lymph node metastasis within individuals with homozygous *HLA-C2*<sup>79</sup>. A previous study by Momot et al. suggested that carrying *2DS2*<sup>+</sup>/*L2*<sup>-</sup> combination is related to a higher risk of scleroderma disease<sup>80</sup>, and similar results were illustrated regarding systemic sclerosis<sup>81</sup>. However, this disagreement with our observations might be due to different mechanisms involved in tumorigenesis and autoimmune disorders. The fact that the difference of genotypes with *iKIRs* > *aKIRs* and CxTx combined with *2DL2* but not *2DS2* remained significant after correction of *p* values implies that these combinations are significantly more strongly associated with lung cancer development. It can also be presumed that the presence of *2DL2* intensifies disease associations.

It is important to highlight the suppressive effects on NK cells suggested to be driven by alveolar macrophages and epithelial lining fluid of the lower respiratory tract<sup>82</sup>. Despite comprising a well-differentiated phenotype, NK cells in lung tissue are exposed to a restricting microenvironment in homeostasis, causing a hypo-functional state of lung NK cells to stimuli in comparison with NK cells of peripheral blood<sup>83,84</sup>. Attenuated killing potency in TME owing to the presence of regulatory T cells (TREGs) and myeloid-derived suppressor cells (MDSCs), limitations of leukocyte infiltration, as well as immunosuppressive factors in lung TME such as adenosine and transforming growth factor  $\beta$  (TGF- $\beta$ ) are yet to be overcome<sup>4,85</sup>. Taken together, it can be speculated that carrying more inhibitory gene content may interfere with NK-mediated immunosurveillance, favoring tumor evasion in the existing suppressor context of lung tissue. On the other end, activating gene content is presumed to restore the functional competence of NK cells, especially in suppressive lung settings.

In conclusion, we report new susceptibility factors and the contribution of *KIR* and *HLA-I* encoding genes to lung cancer risk, highlighting an array of genetic effects and disease setting that regulates NK cell responsiveness. Our results suggest that inherited *KIR* genes and *HLA-I* ligands specifying the educational state of NK cells can modify lung cancer risk. In the current study, *HLA-I* ligands and co-associations of *KIR-HLA* were examined for a limited number of subjects; also an inadequate number of patients with different lung cancer subtypes hindered the evaluation of *KIRs* and *HLA-I* genes impact within subgroups. Thus, larger cohorts assessing the contribution of *KIR-HLA* combinations are needed to confirm these associations. Functional analysis might help extend associations to the potential therapeutic strategies against lung cancer. The unique microenvironment of each lung cancer subtype with a varied composition of immune cells is assumed to affect NK cell characteristics<sup>86</sup>. Further investigations on mechanisms involved in NK cell dysfunction in different subtypes might develop into NK-based immunotherapies in lung cancer.

## Materials and methods

**Study subjects.** A total of 232 patients with lung cancer (comprising NSCLC: squamous cell carcinoma and adenocarcinoma, SCLC subtypes) and 448 healthy controls (HC) from a homogenous population of the southern part of Iran (Fras province) were included in this case-control study. The enrolled group of 232 unrelated lung cancer patients was made up 85.3% of men and 14.7% of women with a mean age of  $64.4 \pm 11.1$ . Healthy controls were comprised 66.5% of men and 33.5% of women with a mean age of  $58.13 \pm 12.66$ . The pathologically confirmed lung cancer cases were recruited from Faghihi hospital, Shiraz University of medical sciences. Age and sex-matched HCs with no Family History of Cancer (FHC) were selected from the Motahari clinic. The demographic and clinical characteristics of lung cancer patients were gathered from medical records (Table 1). Due to the fact that smoking status for a very small portion of our lung cancer patients and HCs was available, stratification of the study population based on smoking status wasn't carried out.

Informed consent from all research participants was obtained, and the study was carried out according to the declaration of Helsinki. This study was reviewed and ethically approved by the Medical Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1398.1110).

**KIR genotyping.** Genomic DNA extraction from whole blood samples was performed using QIAamp DNA Mini Kit (Qiagen, Germany) as detailed in the manufacturer's instructions. The PCR-SSP method was used for genotyping 16 *KIR* genes, and *KIR2DS4* variants (*KIR2DS4fl*: *2DS4* full variant, and *KIR2DS4del*: *2DS4* deleted variant) as previously described<sup>87</sup>. Detailed information on primer sequences, thermal conditions, and the mixture of each reaction are reported in our previous study<sup>59</sup>. Reference DNA samples from the UCLA KIR exchange program provided by Prof. Raja Rajalingam were applied to ensure typing accuracy. An alternative SSP-PCR method was used to confirm the unique and unusual KIR genotyping<sup>88</sup>.

The KIR-binding *HLA-A*, *B*, and *C* ligands of 85 lung cancer patients and 178 HCs were typed using the recently developed direct DNA sequencing method. The strategy includes PCR amplifying exons 2 and 3 using *HLA-A*, *B*, or *C* gene-specific primers and direct sequencing of the segment that encodes the KIR-binding region. The process was accomplished in accordance with the method described by Ashouri et al.<sup>89</sup>.

**Data analysis and statistical methods.** KIR genotypes of the study participants were assigned according to previous studies<sup>88,90</sup>. The genotype AA comprises fixed gene content (*2DL3-2DL1-2DP1-3DL1-2DS4*) surrounded by frameworks. Carriers of AA genotype-related genes were regarded as homozygous AA, and the remaining subjects were considered as Bx genotype carriers which can be heterozygous AB or homozygous BB. The KIR genotype ID was obtained using the allele frequency database (<http://www.allelefrequencies.net/>) for all participants.

Based on the linkage disequilibrium, two frequently occurring clusters that include distinct sets of B-haplotype-specific KIR genes have been identified<sup>91</sup>. C4 linkage group comprising *KIR2DS2-2DL2-2DS3-2DL5B* genes is located in the centromeric region of the KIR complex, while the T4 linkage group contains

*KIR3DS1-2DL5A-2DS5-2DS1* genes located at the telomeric region of the complex. Concerning the presence or absence of C4 and T4 linkage groups, The Bx genotype carriers were further divided into the following four subsets: C4Tx, CxT4, C4T4, and CxTx<sup>89</sup>. The frequency of C4 and T4 gene clusters were defined by subsequent formulas: C4 = nC4Tx + nC4T4, and T4 = nCxT4 + nC4T4 (n: number of individuals with a particular subset within each group).

The percentage of *KIR* genes in both study groups was indicated by direct counting (number of subjects positive for the gene divided by the number of subjects per population × 100). Differences in the distribution of each *KIR* gene, genotypes, KIR-binding HLA ligands, and KIR-HLA pairs between lung cancer patients and HCs were estimated by two-tailed Fisher Exact probability (*p*) test using SPSS (IBM, US) version 16.0 and Items with *p* < 0.05 were considered as statistically significant. Moreover, the Odds ratio (OR) and 95% Confidence Intervals (CI) were calculated to assess the magnitude of associations. The method expounded by Svejgaard and Ryde<sup>92</sup> was applied to identify the combined effect of the lung cancer susceptibility factors CxTx<sup>+</sup>/2DL2<sup>+</sup> and CxTx<sup>+</sup>/2DS2<sup>+</sup>. *p* values regarding the association of genotypes with certain number of genes (*aKIRs* > *iKIRs*, *iKIRs* > *aKIRs*, *aKIRs* > 4, *iKIRs* > 4) were corrected using  $P_n = 1 - (1 - P)^n$ , where n represents the number of comparisons<sup>92</sup>.

**Ethical approval.** Ethical approval of the research was confirmed by the Medical Ethics Committee of Shiraz University of Medical Sciences [IR. SUMS.REC.1398.1110].

### Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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### References

1. Dela Cruz, C. S., Tanoue, L. T. & Matthay, R. A. Lung cancer: Epidemiology, etiology, and prevention. *Clin. Chest Med.* **32**, 605–644. <https://doi.org/10.1016/j.ccm.2011.09.001> (2011).
2. Chen, Z., Fillmore, C. M., Hammerman, P. S., Kim, C. F. & Wong, K. K. Non-small-cell lung cancers: A heterogeneous set of diseases. *Nat. Rev. Cancer* **14**, 535–546. <https://doi.org/10.1038/nrc3775> (2014).
3. Borish, L. The immunology of asthma: Asthma phenotypes and their implications for personalized treatment. *Ann. Allergy Asthma Immunol.* **117**, 108–114. <https://doi.org/10.1016/j.anaai.2016.04.022> (2016).
4. Morvan, M. G. & Lanier, L. L. NK cells and cancer: You can teach innate cells new tricks. *Nat. Rev. Cancer* **16**, 7–19. <https://doi.org/10.1038/nrc.2015.5> (2016).
5. Smyth, M. J., Hayakawa, Y., Takeda, K. & Yagita, H. New aspects of natural-killer-cell surveillance and therapy of cancer. *Nat. Rev. Cancer* **2**, 850–861. <https://doi.org/10.1038/nrc928> (2002).
6. Cong, J. & Wei, H. Natural killer cells in the lungs. *Front. Immunol.* **10**, 1416. <https://doi.org/10.3389/fimmu.2019.01416> (2019).
7. Cong, J. *et al.* Dysfunction of natural killer cells by FBP1-induced inhibition of glycolysis during lung cancer progression. *Cell Metab.* **28**, 243–255.e5. <https://doi.org/10.1016/j.cmet.2018.06.021> (2018).
8. Takeda, K. *et al.* IFN- $\gamma$  production by lung NK cells is critical for the natural resistance to pulmonary metastasis of B16 melanoma in mice. *J. Leukoc. Biol.* **90**, 777–785. <https://doi.org/10.1189/jlb.0411208> (2011).
9. Takanami, I., Takeuchi, K. & Giga, M. The prognostic value of natural killer cell infiltration in resected pulmonary adenocarcinoma. *J. Thorac. Cardiovasc. Surg.* **121**, 1058–1063. <https://doi.org/10.1067/mtc.2001.113026> (2001).
10. Villegas, F. R. *et al.* Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. *Lung Cancer* **35**, 23–28. [https://doi.org/10.1016/s0169-5002\(01\)00292-6](https://doi.org/10.1016/s0169-5002(01)00292-6) (2002).
11. Carrega, P. *et al.* Natural killer cells infiltrating human nonsmall-cell lung cancer are enriched in CD56 bright CD16(-) cells and display an impaired capability to kill tumor cells. *Cancer* **112**, 863–875. <https://doi.org/10.1002/cncr.23239> (2008).
12. Platonova, S. *et al.* Profound coordinated alterations of intratumoral NK cell phenotype and function in lung carcinoma. *Cancer Res.* **71**, 5412–5422. <https://doi.org/10.1158/0008-5472.can-10-4179> (2011).
13. Bruno, A. *et al.* The proangiogenic phenotype of natural killer cells in patients with non-small cell lung cancer. *Neoplasia* **15**, 133–142. <https://doi.org/10.1593/neo.121758> (2013).
14. Djaoud, Z. & Parham, P. HLAs, TCRs, and KIRs, a triumvirate of human cell-mediated immunity. *Annu. Rev. Biochem.* **89**, 717–739. <https://doi.org/10.1146/annurev-biochem-011520-102754> (2020).
15. Olcese, L. *et al.* Human and mouse killer-cell inhibitory receptors recruit PTP1C and PTP1D protein tyrosine phosphatases. *J. Immunol.* **156**, 4531–4534 (1996).
16. Parham, P. MHC class I molecules and KIRs in human history, health and survival. *Nat. Rev. Immunol.* **5**, 201–214. <https://doi.org/10.1038/nri1570> (2005).
17. Pyo, C. W. *et al.* Different patterns of evolution in the centromeric and telomeric regions of group A and B haplotypes of the human killer cell Ig-like receptor locus. *PLoS ONE* **5**, e15115. <https://doi.org/10.1371/journal.pone.0015115> (2010).
18. Norman, P. J. *et al.* Meiotic recombination generates rich diversity in NK cell receptor genes, alleles, and haplotypes. *Genome Res.* **19**, 757–769. <https://doi.org/10.1101/gr.085738.108> (2009).
19. Ordóñez, D. *et al.* Duplication, mutation and recombination of the human orphan gene KIR2DS3 contribute to the diversity of KIR haplotypes. *Genes Immun.* **9**, 431–437. <https://doi.org/10.1038/gene.2008.34> (2008).
20. Single, R. M., Martin, M. P., Meyer, D., Gao, X. & Carrington, M. Methods for assessing gene content diversity of KIR with examples from a global set of populations. *Immunogenetics* **60**, 711–725. <https://doi.org/10.1007/s00251-008-0331-1> (2008).
21. Guethlein, L. A., Norman, P. J., Hilton, H. G. & Parham, P. Co-evolution of MHC class I and variable NK cell receptors in placental mammals. *Immunol. Rev.* **267**, 259–282. <https://doi.org/10.1111/imr.12326> (2015).
22. Gardiner, C. M. *et al.* Different NK cell surface phenotypes defined by the DX9 antibody are due to KIR3DL1 gene polymorphism. *J. Immunol.* **166**, 2992–3001. <https://doi.org/10.4049/jimmunol.166.5.2992> (2001).
23. Parham, P., Norman, P. J., Abi-Rached, L. & Guethlein, L. A. Variable NK cell receptors exemplified by human KIR3DL1/S1. *J. Immunol.* **187**, 11–19. <https://doi.org/10.4049/jimmunol.0902332> (2011).
24. Rajalingam, R. Diversity of killer cell immunoglobulin-like receptors and disease. *Clin. Lab. Med.* **38**, 637–653. <https://doi.org/10.1016/j.cll.2018.08.001> (2018).
25. Dębska-Zielkowska, J. *et al.* KIR receptors as key regulators of NK cells activity in health and disease. *Cells* **10**, 1777. <https://doi.org/10.3390/cells10071777> (2021).

26. Gumperz, J. E., Litwin, V., Phillips, J. H., Lanier, L. L. & Parham, P. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NK1, a putative HLA receptor. *J. Exp. Med.* **181**, 1133–1144. <https://doi.org/10.1084/jem.181.3.1133> (1995).
27. Döhning, C., Scheidegger, D., Samaridis, J., Cella, M. & Colonna, M. A human killer inhibitory receptor specific for HLA-A1,2. *J. Immunol.* **156**, 3098–3101 (1996).
28. Martin, M. P. *et al.* Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat. Genet.* **39**, 733–740. <https://doi.org/10.1038/ng2035> (2007).
29. Martin, M. P. *et al.* Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat. Genet.* **31**, 429–434. <https://doi.org/10.1038/ng934> (2002).
30. Cheung, J. H., Gudme, C. N., Hsu, K. C., Selvakumar, A. & Dupont, B. KIR2DS1-positive NK cells mediate alloresponse against the C2 HLA-KIR ligand group in vitro. *J. Immunol.* **179**, 854–868. <https://doi.org/10.4049/jimmunol.179.2.854> (2007).
31. Biassoni, R. *et al.* Role of amino acid position 70 in the binding affinity of p50.1 and p58.1 receptors for HLA-Cw4 molecules. *Eur. J. Immunol.* **27**, 3095–3099. <https://doi.org/10.1002/eji.1830271203> (1997).
32. Stewart, C. A. *et al.* Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 13224–13229. <https://doi.org/10.1073/pnas.0503594102> (2005).
33. Dubreuil, L., Chevallier, P., Retière, C. & Gagne, K. Relevance of polymorphic KIR and HLA class I genes in NK-cell-based immunotherapies for adult leukemic patients. *Cancers* **13**, 3767. <https://doi.org/10.3390/cancers13153767> (2021).
34. Anfossi, N. *et al.* Human NK cell education by inhibitory receptors for MHC class I. *Immunity* **25**, 331–342. <https://doi.org/10.1016/j.immuni.2006.06.013> (2006).
35. Kim, S. *et al.* HLA alleles determine differences in human natural killer cell responsiveness and potency. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 3053–3058. <https://doi.org/10.1073/pnas.0712229105> (2008).
36. Pando, M. J., Gardiner, C. M., Gleimer, M., McQueen, K. L. & Parham, P. The protein made from a common allele of KIR3DL1 (3DL1\*004) is poorly expressed at cell surfaces due to substitution at positions 86 in Ig domain 0 and 182 in Ig domain 1. *J. Immunol.* **171**, 6640–6649. <https://doi.org/10.4049/jimmunol.171.12.6640> (2003).
37. Fernandez, M. I. *et al.* Maturation of paneth cells induces the refractory state of newborn mice to Shigella infection. *J. Immunol.* **180**, 4924–4930. <https://doi.org/10.4049/jimmunol.180.7.4924> (2008).
38. Bari, R. *et al.* Significant functional heterogeneity among KIR2DL1 alleles and a pivotal role of arginine 245. *Blood* **114**, 5182–5190. <https://doi.org/10.1182/blood-2009-07-231977> (2009).
39. Béziat, V., Hilton, H. G., Norman, P. J. & Traherne, J. A. Deciphering the killer-cell immunoglobulin-like receptor system at super-resolution for natural killer and T-cell biology. *Immunology* **150**, 248–264. <https://doi.org/10.1111/imm.12684> (2017).
40. Bossé, Y. & Amos, C. I. A decade of GWAS results in lung cancer. *Cancer. Epidemiol. Biomarkers. Prev.* **27**, 363–379. <https://doi.org/10.1158/1055-9965.EPI-16-0794> (2018).
41. Musolf, A. M. *et al.* Familial lung cancer: A brief history from the earliest work to the most recent studies. *Genes* **8**, E36. <https://doi.org/10.3390/genes8010036> (2017).
42. Li, Y. *et al.* The association of HLA/KIR genes with non-small cell lung cancer (adenocarcinoma) in a Han Chinese population. *J. Cancer* **10**, 4731–4738. <https://doi.org/10.7150/jca.33566> (2019).
43. Yu, H. *et al.* 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**, 279–285. <https://doi.org/10.3892/mco.2016.1106> (2017).
44. Wiśniewski, A. *et al.* KIR2DL2/S2 and HLA-C C1C1 genotype is associated with better response to treatment and prolonged survival of patients with non-small cell lung cancer in a Polish Caucasian population. *Hum. Immunol.* **73**, 927–931. <https://doi.org/10.1016/j.humimm.2012.07.323> (2012).
45. Al Omar, S. *et al.* Associations between genes for killer immunoglobulin-like receptors and their ligands in patients with solid tumors. *Hum. Immunol.* **71**, 976–981. <https://doi.org/10.1016/j.humimm.2010.06.019> (2010).
46. Seo, J. S., Kim, A., Shin, J. Y. & Kim, Y. T. Comprehensive analysis of the tumor immune micro-environment in non-small cell lung cancer for efficacy of checkpoint inhibitor. *Sci. Rep.* **8**, 14576. <https://doi.org/10.1038/s41598-018-32855-8> (2018).
47. Abruzzese, M. P. *et al.* Inhibition of bromodomain and extra-terminal (BET) proteins increases NKG2D ligand MICA expression and sensitivity to NK cell-mediated cytotoxicity in multiple myeloma cells: Role of cMYC-IRF4-miR-125b interplay. *J. Hematol. Oncol.* **9**, 134. <https://doi.org/10.1186/s13045-016-0362-2> (2016).
48. Fionda, C. *et al.* Inhibition of glycogen synthase kinase-3 increases NKG2D ligand MICA expression and sensitivity to NK cell-mediated cytotoxicity in multiple myeloma cells: Role of STAT3. *J. Immunol.* **190**, 6662–6672. <https://doi.org/10.4049/jimmunol.1201426> (2013).
49. Fionda, C. *et al.* Heat shock protein-90 inhibitors increase MHC class I-related chain A and B ligand expression on multiple myeloma cells and their ability to trigger NK cell degranulation. *J. Immunol.* **183**, 4385–4394. <https://doi.org/10.4049/jimmunol.0901797> (2009).
50. Hogg, S. J. *et al.* BET-bromodomain inhibitors engage the host immune system and regulate expression of the immune checkpoint ligand PD-L1. *Cell Rep.* **18**, 2162–2174. <https://doi.org/10.1016/j.celrep.2017.02.011> (2017).
51. Shi, J. *et al.* Bortezomib down-regulates the cell-surface expression of HLA class I and enhances natural killer cell-mediated lysis of myeloma. *Blood* **111**, 1309–1317. <https://doi.org/10.1182/blood-2007-03-078535> (2008).
52. Markasz, L. *et al.* Effect of frequently used chemotherapeutic drugs on the cytotoxic activity of human natural killer cells. *Mol. Cancer Ther.* **6**, 644–654. <https://doi.org/10.1158/1535-7163.mct-06-0358> (2007).
53. Sako, T. *et al.* Cellular immune profile in patients with non-small cell lung cancer after weekly paclitaxel therapy. *Acta Oncol.* **43**, 15–19. <https://doi.org/10.1080/02841860310016226> (2004).
54. Marin, M. L. C. *et al.* Inhibitory KIR2DL2 Gene: Risk for deep endometriosis in euro-descendants. *Reprod. Sci.* **28**, 291–304. <https://doi.org/10.1007/s43032-020-00255-x> (2021).
55. Verheyden, S., Bernier, M. & Demanet, C. Identification of natural killer cell receptor phenotypes associated with leukemia. *Leukemia* **18**, 2002–2007. <https://doi.org/10.1038/sj.leu.2403525> (2004).
56. Barani, S., Khademi, B. & Ghaderi, A. KIR2DS4, KIR2DL2, and KIR2DS4del are linked with basaloid tumors, lymph node metastasis, advanced stage and metastatic risk in head and neck squamous cell carcinoma. *Exp. Mol. Pathol.* **112**, 104345. <https://doi.org/10.1016/j.yexmp.2019.104345> (2020).
57. Kandilarova, S. M. *et al.* The influence of HLA and KIR genes on malignant melanoma development and progression. *Arch. Immunol. Ther. Exp. (Warsz)* **64**, 73–81. <https://doi.org/10.1007/s00005-016-0437-3> (2016).
58. Naumova, E. *et al.* Genetic polymorphism of NK receptors and their ligands in melanoma patients: Prevalence of inhibitory over activating signals. *Cancer Immunol. Immunother.* **54**, 172–178. <https://doi.org/10.1007/s00262-004-0575-z> (2005).
59. Hematian Larki, M., Barani, S., Talei, A. R. & Ghaderi, A. Diversity of KIRs in invasive breast cancer patients and healthy controls along with the clinical significance in ER/PR/HER2+ patients. *Genes Immun.* **21**, 380–389. <https://doi.org/10.1038/s41435-020-00117-1> (2020).
60. He, Y. & Tian, Z. NK cell education via nonclassical MHC and non-MHC ligands. *Cell Mol. Immunol.* **14**, 321–330. <https://doi.org/10.1038/cmi.2016.26> (2017).
61. Perea, F. *et al.* The absence of HLA class I expression in non-small cell lung cancer correlates with the tumor tissue structure and the pattern of T cell infiltration. *Int. J. Cancer* **140**, 888–899. <https://doi.org/10.1002/ijc.30489> (2017).

62. Perea, F. *et al.* HLA class I loss and PD-L1 expression in lung cancer: Impact on T-cell infiltration and immune escape. *Oncotarget* **9**, 4120–4133. <https://doi.org/10.18632/oncotarget.23469> (2018).
63. Rajagopalan, S. & Long, E. O. The direct binding of a p58 killer cell inhibitory receptor to human histocompatibility leukocyte antigen (HLA)-Cw4 exhibits peptide selectivity. *J. Exp. Med.* **185**, 1523–1528. <https://doi.org/10.1084/jem.185.8.1523> (1997).
64. Carrillo-Bustamante, P., de Boer, R. J. & Keşmir, C. Specificity of inhibitory KIRs enables NK cells to detect changes in an altered peptide environment. *Immunogenetics* **70**, 87–97. <https://doi.org/10.1007/s00251-017-1019-1> (2018).
65. Thiruchelvam-Kyle, L. *et al.* The activating human NK cell receptor KIR2DS2 recognizes a  $\beta$ 2-microglobulin-independent ligand on cancer cells. *J. Immunol.* **198**, 2556–2567. <https://doi.org/10.4049/jimmunol.1600930> (2017).
66. Katz, G. *et al.* MHC class I-independent recognition of NK-activating receptor KIR2DS4. *J. Immunol.* **173**, 1819–1825. <https://doi.org/10.4049/jimmunol.173.3.1819> (2004).
67. Fauriat, C., Ivarsson, M. A., Ljunggren, H. G., Malmberg, K. J. & Michaëlsson, J. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. *Blood* **115**, 1166–1174. <https://doi.org/10.1182/blood-2009-09-245746> (2010).
68. Carlomagno, S. *et al.* KIR3DS1-mediated recognition of HLA- $\ast$ B51: Modulation of KIR3DS1 responsiveness by self HLA-B alleles and effect on NK cell licensing. *Front. Immunol.* **8**, 581. <https://doi.org/10.3389/fimmu.2017.00581> (2017).
69. Garcia-Beltran, W. F. *et al.* Open conformers of HLA-F are high-affinity ligands of the activating NK-cell receptor KIR3DS1. *Nat. Immunol.* **17**, 1067–1074. <https://doi.org/10.1038/ni.3513> (2016).
70. Kiani, Z. *et al.* HLA-F on HLA-Null 721.221 cells activates primary NK cells expressing the activating killer Ig-like receptor KIR3DS1. *J. Immunol.* **201**, 113–123. <https://doi.org/10.4049/jimmunol.1701370> (2018).
71. Lin, A. *et al.* HLA-F expression is a prognostic factor in patients with non-small-cell lung cancer. *Lung Cancer* **74**, 504–509. <https://doi.org/10.1016/j.lungcan.2011.04.006> (2011).
72. Moesta, A. K. & Parham, P. Diverse functionality among human NK cell receptors for the C1 epitope of HLA-C: KIR2DS2, KIR2DL2, and KIR2DL3. *Front. Immunol.* **3**, 336. <https://doi.org/10.3389/fimmu.2012.00336> (2012).
73. Cassidy, S. *et al.* Peptide selectivity discriminates NK cells from KIR2DL2- and KIR2DL3-positive individuals. *Eur. J. Immunol.* **45**, 492–500. <https://doi.org/10.1002/eji.201444613> (2015).
74. Fadda, L. *et al.* Peptide antagonism as a mechanism for NK cell activation. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 10160–10165. <https://doi.org/10.1073/pnas.0913745107> (2010).
75. Barani, S., Khademi, B., Ashouri, E. & Ghaderi, A. KIR2DS1, 2DS5, 3DS1 and KIR2DL5 are associated with the risk of head and neck squamous cell carcinoma in Iranians. *Hum. Immunol.* **79**, 218–223. <https://doi.org/10.1016/j.humimm.2018.01.012> (2018).
76. Barani, S., Hosseini, S. V. & Ghaderi, A. 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. *Immunobiology* **224**, 681–686. <https://doi.org/10.1016/j.imbio.2019.06.002> (2019).
77. Barani, S., Taghipour, M. & Ghaderi, A. Positive association of Bx genotype, KIR2L5, KIR2DS5 and full-length KIR2DS4 with the risk of meningioma. *Immunobiology* **225**, 151900. <https://doi.org/10.1016/j.imbio.2019.151900> (2020).
78. Al Omar, S. Y., Marshall, E., Middleton, D. & Christmas, S. E. Increased killer immunoglobulin-like receptor expression and functional defects in natural killer cells in lung cancer. *Immunology* **133**, 94–104. <https://doi.org/10.1111/j.1365-2567.2011.03415.x> (2011).
79. Campillo, J. A. *et al.* KIR gene variability in cutaneous malignant melanoma: Influence of KIR2D/HLA-C pairings on disease susceptibility and prognosis. *Immunogenetics* **65**, 333–343. <https://doi.org/10.1007/s00251-013-0682-0> (2013).
80. Momot, T. *et al.* Association of killer cell immunoglobulin-like receptors with scleroderma. *Arthritis Rheum.* **50**, 1561–1565. <https://doi.org/10.1002/art.20216> (2004).
81. Salim, P. H. *et al.* Killer cell immunoglobulin-like receptor (KIR) genes in systemic sclerosis. *Clin. Exp. Immunol.* **160**, 325–330. <https://doi.org/10.1111/j.1365-2249.2010.04095.x> (2010).
82. Robinson, B. W., Pinkston, P. & Crystal, R. G. Natural killer cells are present in the normal human lung but are functionally impotent. *J. Clin. Invest.* **74**, 942–950. <https://doi.org/10.1172/JCI111513> (1984).
83. Marquardt, N. *et al.* Human lung natural killer cells are predominantly comprised of highly differentiated hypofunctional CD69-CD56dim cells. *J. Allergy. Clin. Immunol.* **139**, 1321–1330.e4. <https://doi.org/10.1016/j.jaci.2016.07.043> (2017).
84. Wang, J. *et al.* Lung natural killer cells in mice: Phenotype and response to respiratory infection. *Immunology* **137**, 37–47. <https://doi.org/10.1111/j.1365-2567.2012.03607.x> (2012).
85. Chiossone, L., Dumas, P. Y., Vienne, M. & Vivier, E. Natural killer cells and other innate lymphoid cells in cancer. *Nat. Rev. Immunol.* **18**, 671–688. <https://doi.org/10.1038/s41577-018-0061-z> (2018).
86. Björkström, N. K., Ljunggren, H. G. & Michaëlsson, J. Emerging insights into natural killer cells in human peripheral tissues. *Nat. Rev. Immunol.* **16**, 310–320. <https://doi.org/10.1038/nri.2016.34> (2016).
87. Vilches, C., Castaño, J., Gómez-Lozano, N. & Estefanía, E. Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. *Tissue Antigens* **70**, 415–422. <https://doi.org/10.1111/j.1399-0039.2007.00923.x> (2007).
88. Du, Z., Gjertson, D. W., Reed, E. F. & Rajalingam, R. Receptor-ligand analyses define minimal killer cell Ig-like receptor (KIR) in humans. *Immunogenetics* **59**, 1–15. <https://doi.org/10.1007/s00251-006-0168-4> (2007).
89. Ashouri, E. *et al.* Coexistence of inhibitory and activating killer-cell immunoglobulin-like receptors to the same cognate HLA-C2 and Bw4 ligands confer breast cancer risk. *Sci. Rep.* **11**, 7932. <https://doi.org/10.1038/s41598-021-86964-y> (2021).
90. Ashouri, E., Farjadian, S., Reed, E. F., Ghaderi, A. & Rajalingam, R. KIR gene content diversity in four Iranian populations. *Immunogenetics* **61**, 483–492. <https://doi.org/10.1007/s00251-009-0378-7> (2009).
91. Du, Z., Sharma, S. K., Spellman, S., Reed, E. F. & Rajalingam, R. KIR2DL5 alleles mark certain combination of activating KIR genes. *Genes Immun.* **9**, 470–480. <https://doi.org/10.1038/gene.2008.39> (2008).
92. Svejgaard, A. & Ryder, L. P. HLA and disease associations: Detecting the strongest association. *Tissue Antigens* **43**, 18–27. <https://doi.org/10.1111/j.1399-0039.1994.tb02291.x> (1994).

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## Author contributions

M.H.L. and E.A. performed K.I.R. and H.L.A. ligands typing. M.H.L. prepared the first draft of manuscript. S.B. contributed to the statistical analysis and edited the first draft of the manuscript. S.M.A.G. provided samples and clinical data. A.G. and R.R. designed the study, performed the interpretation, and edited the manuscript final version. The paper was reviewed and approved by all authors.

## Competing interests

The authors declare no competing interests.

### Additional information

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