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ORIGINAL ARTICLE

The Influence of HLA and KIR Genes on Malignant Melanoma Development and Progression

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Abstract Many studies have described the role of killer immunoglobulin-like receptors (KIRs) and their cognate human leukocyte antigen (HLA) class I ligands in the immune protection against melanoma, but the effect of these markers on intra-individual variations in tumor development and progression has remained less clear. We performed KIR, HLA, and KIR/ligand analysis in 283 patients with malignant melanoma in order to evaluate their integrated influence on disease stage and progression. The patients were grouped according to AJCC staging, histological type of the primary tumor, progression, and survival rate. Analysis of HLA class I alleles revealed positive association of HLA-C*14 (Pc = 0.026, OR = 5.99) and negative association of HLA-C*02 (Pc = 0.026, OR = 0.43) with the disease. Decreased frequency of KIR2DS5 was observed in patients with rapid progression, as compared to those with slow progression. KIR BB genotype was prevalent in patients with metastasis ($p = 0.004$, OR = 0.025). KIR AA genotype was nearly twice as frequent in rapidly progressive cases, but without statistical relevance ($p = 0.055$, OR = 2.6). Significantly increased frequency of KIR2DL2 in the presence of C1 ligand (strong inhibition) was found in patients with AJCC III and IV, as compared to individuals with AJCC I stage ($p = 0.045$, OR = 1.93). In summary, our data imply that KIR/ligand gene content in patients could modulate the disease course towards unfavorable tumor behavior.

Keywords Malignant melanoma · Progression · Inhibitory killer immunoglobulin-like receptors (KIR) · Human leukocyte antigens (HLA)

Introduction

It has been known that natural killer (NK) cells have the ability to kill tumor cell lines and the cytotoxic effect has been demonstrated mainly in vitro (Carlsten et al. 2009). Apart from that, their role in controlling the processes of tumor progression and metastasis formation has been an object of intensive studies in the past few years. It has been demonstrated in mice models that NK cells are recruited to regional lymph nodes and participate in the suppression of tumor metastasis formation (Chen et al. 2005). Furthermore, recent work has demonstrated that tumor-infiltrated lymph nodes from melanoma patients are enriched with cytotoxic NK cells. These cells express different activating receptors and chemokine receptors and when activated by IL-2 or IL-15 cytokines they could efficiently lyse metastatic melanoma cells (Messaoudene et al. 2014). An expansion of particular NK subsets expressing the receptors for CXCL8, CCL2, and IL-6 in melanoma-infiltrated lymph nodes has also been shown to generate anti-tumor microenvironment (Ali et al. 2014).

NK cells can be activated through multitude signals, produced by diverse inhibitory and activating receptors. The detection of tumor cells that have lost expression of one or multiple human leukocyte antigens (HLA) class I is accomplished by the NK cells through HLA-A, -B, and -C allotype-specific inhibitory receptors, known as inhibitory killer

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immunoglobulin-like receptors (KIRs) (Parham 2005). The inhibitory KIRs with three extracellular immunoglobulin domains (KIR3DL) interact with HLA-A or -B alleles. HLA-A3 and -A11 are bound by KIR3DL2 (Hansasuta et al. 2004) and KIR3DL1 binds HLA-B molecules carrying the Bw4 motif. The inhibitory KIRs, having two extracellular domains (KIR2DL), demonstrate a similar action with HLA-C allotypes. KIR2DL1 interacts with group 2 HLA-C specificities, while the interplay of the KIR2DL2 and KIR2DL3 is with group 1 HLA-C (Gumperz et al. 1997). Since the HLA-C ligands for inhibitory KIR are present in all individuals, it is regarded as the main ligand for KIRs. Two distinct KIR haplotypes have resulted from the presence or absence of particular KIR genes commonly designated as A and B. KIR2DL1, 2DL3, 3DL1, and 2DS4 genes are present in haplotype A, while one or more of the following genes are part of B haplotype: KIR2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, and 3DS1 (Bashirova et al. 2006). In agreement with the "missing-self" hypothesis, cells that downregulate or lack HLA class I expression become susceptible to NK cell-mediated lysis. However, an important fact is that efficient killing is also dependent on activating receptors binding to particular types of ligands on the target cells. Thus, NK cell activity is controlled by a balance of both activating and inhibitory signals. The knowledge available on KIRs and their ligands led to generation of some genetic studies on KIR/HLA ligand association with malignancies, including one of the most immunogenic tumors—malignant melanoma (MM) (Naumova et al. 2007). Studies have shown a number of statistically significant associations between frequencies of KIR genes and their ligands in malignant formations (Boyton and Altmann 2007; Campillo et al. 2013). Most reports in this field, however, have focused on the role of KIR and HLA associations in tumor predisposition/protection, while their effect on intra-individual variation of tumor development and progression has remained less clear. The purpose of this study was to carry out KIR, HLA, and KIR/ligand analysis in a cohort of clinically defined patients with MM, in order to evaluate their compound effect on the occurrence, stage, and progression of the disease. As NK cells form the first line of immune defense against tumors, investigation of the genetic polymorphism determining NK cell function in different stages and types of melanoma may provide further understanding of tumor immune escape and metastatic progression mechanisms.

Materials and Methods

Population Under Study

Blood samples were collected from patients with Europid origin at the Medical University, Mannheim, Germany with the approval of the local ethics committee and the patients' informed consent provided. Material was obtained from a total of 283 patients, 133 females and 150 males, with different melanoma location types (skin, mucosa, eyes). The average age of the group was 59.69 ± 14.99 years (28–85 years). They were first diagnosed with the disease in the period 1987–2005. The patients' clinical outcome was followed for a different period of time up to the year 2007. At the time of the first examination the patients were clinically characterized by the American Joint Committee on Cancer (AJCC; data available for 265 patients). For the present study the melanoma patients were divided into two groups, according to the AJCC staging system: patients with localized, primary disease (clinical stages I and II) and patients with in regional lymph nodes and various distant organs (clinical stage III and IV). The histological type of the primary tumor in each patient was defined as belonging to one of the following groups: superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma, and amelanotic melanoma. The tumor progression time was followed in 116 of the patients presenting with AJCCI/II stage at the time of first admission. Rapid progression of the disease was assigned to patients advancing from AJCCI/II to AJCCIII/IV within 18 months of diagnosis. The relevant cut-off period for slow progression was more than 18 months. Survival data were available for 270 patients. Eighty-two of them died in the first 5 years of diagnosis, 106 were alive by the date of the last contact and 82 survived for more than 5 years. Data from the Allele Frequency Net Database on the HLA class I and KIR allele distribution among healthy subjects from the German population were used for a comparative analysis (Becker et al. 2003; Gonzalez-Galarza et al. 2011).

KIR Genotyping

DNA was isolated from cryopreserved peripheral blood cell samples using the DNA Blood MiniKit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Low resolution HLA-A and -B genotyping was carried out at the Institute for Transfusion Medicine Mannheim (Mannheim, Germany) using routine PCR-based methods. HLA-C genotyping was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP). The presence of HLA-C group 1 and group 2 alleles was determined accordingly. KIR genotyping (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, 3DP1, 2DL5, 2DS4, and 3DP1) was performed by the PCR-SSP method using a PEL-FREEZ KIR Genotyping Kit (Dynal Biotech, Brown Deer, WI, USA). On the basis of individual KIR profile, the patient's KIR genotypes (AA, AB, BB) were identified.

KIR genotyping results were available for this analysis in 278 cases (98% of cases). KIR/ligand genotype frequencies in different patient groups (AJCC stage, rapid and slow progressions, patients with survival rate either for more or less than 5 years, histological type, and tumor location) were determined by direct counting. Comparison of numbers of given allele in each group studied was performed, applying the χ^2 or Fisher's exact test. p values were corrected for multiple comparisons.

Results

HLA Class I Associations

The available data on the relationship between human leukocyte antigen and cutaneous MM susceptibility or prognosis are controversial and the effect of HLA class I expression and polymorphism on melanoma progression has yet to be established. To test whether polymorphic variants in NK receptors or their ligands contribute to the development of melanoma or not, we first focused on the HLA class I antigens. The most frequent HLA-A alleles found in patients with MM and healthy subjects were A*02, *01, *03. HLA B*40, *07, *44; C*07, *04, *03 were more frequent in patients, while B*07, *08, *40 and C*07, *03, *02 were prevalent in controls (Table 1). Comparison of HLA class I distribution showed statistically decreased frequency of C*02 allele (Pc = 0.026, OR = 0.43) and increased frequency of C*14 allele (Pc = 0.026, OR = 5.99) in MM patients, as compared to healthy Germans. No association of HLA-A and -B loci with the disease was found, although a trend towards a decreased B*55 frequency was noticed in patients. The analysis of HLA class I distribution regarding tumor type and progression revealed no relevance.

KIR Frequencies

Further in our study we analyzed DNA samples of melanoma patients, in order to look for an association of a particular KIR with disease development. KIR genotypes were compiled from KIR gene-specific and allele-specific typing and assigned as AA, AB, or BB, based on those previously defined from haplotype segregation in families (Uhrberg et al. 1997). For 277 melanoma patients the KIR gene content was used successfully to infer group A and B KIR haplotypes and to assign each person to one of three genotypes. Individuals possessing genes of group A KIR haplotypes (3DL3, 2DL1, 2DL3, 2DL4, 3DL1, 2DS4 and 3DL2) only were considered to be homozygous for group A haplotypes and assigned the KIR genotype AA. All the other individuals had one or more B haplotype-specific genes (2DS1, 2DS2, 2DS3, 2DS5, 3DS1 and 2DL5) and thus they either had one (AB heterozygotes) or two (BB homozygotes) B haplotypes. Of all melanoma patients, 81 (29.2%) were homozygous for A haplotypes, 26 (9.4%) were homozygous for B haplotypes, and 170 (61.4%) were AB heterozygotes. On analyzing the genotype distribution among the clinically defined groups, the KIR AA genotype was found to be nearly twice as frequent in rapidly progressive melanoma, as compared to the slowly progressive one (Table 2). On the other hand, BB genotype was prevalent in patients with metastatic involvement (AJCC III/IV)—15.7% versus primary tumor carriers (AJCCI/II)—4.5% ($p = 0.004$, OR = 0.25; Table 2).

The results of the KIR gene distribution in patients (data available for 278 subjects) revealed that gene frequencies ranged from 25.1% (2DL5B*002/004) to 100% (2DL4, 3DL2, 3DL3, 3DP1). 2DL5A*001/005, 2DL5B*002/004, 2DL5B*003, 2DS4 001/002, 003/006, and 3DP1 001/2/4, 003 allele variant was determined in patients' samples, but there were no data available for these genes in healthy Germans. The most frequent KIRs were KIR2DL1, 2DL3, 3DL1, and 2DS4, followed by 2DS2 and 2DL2 (data not presented). The remainder (KIR2DS1, 2DS3, 3DS1) were found in less than half of the samples. The comparison of KIR polymorphisms showed no difference between cancer patients and healthy German subjects for those genes where data were available. The estimated KIR gene distribution among different clinically defined groups was similar, with the exception of a decreased frequency of 2DS5 (20%) observed in rapidly progressive patients (advancing from AJCCI/II to AJCCIII/IV within 18 months of diagnosis), as compared to slowly progressive ones, but without statistical significance (Table 3).

Distribution of HLA-C, Bw4, and HLAA*03/*11 Ligands

Since KIRs recognize HLA-C molecules as ligands depending on the structural basis of HLA-C molecules, the evaluation of the HLA-C distribution among the clinical groups was crucial. Polymorphic HLA-C alleles can be grouped provisionally into "C1" or "C2" according to the presence of asparagine (Asn) or lysine (Lys) at position 80 of HLA-C α-region (Boyington and Sun 2002). To test whether patients with different disease characteristics differed in the genomic representation of the presumable KIR ligands, we performed comparative analysis based on presence of group 1 or group 2 HLA-C ligands. Most frequent among all melanoma patients was the heterozygous HLA-$C^{Asn80+Lys80}$ (C1 + C2) profile—48.5%, followed by HLA-C^{Asn80} (C1)—37.1% and HLA-C^{Lys80} (C2)—14.4% carriers. The frequency of Bw4 ligand among patients was 0.043, or 109 individuals carried this

Table 1 HLA class I frequencies and number of alleles in melanoma patients and healthy controls from German population

Allele	Patients ($n = 283$)		Controls ($n = 174$)		p	Pc*	OR
	Frequency	Number of alleles	Frequency	Number of alleles			
A*01	0.1850	104	0.1440	50			
A*02	0.2638	149	0.2820	98			
A*03	0.1319	75	0.1540	54			
A*11	0.0433	25	0.0440	15			
A*23	0.0217	12	0.0170	6			
A*24	0.1122	63	0.1010	35			
A*25	0.0217	12	0.0240	8			
A*29	0.0236	13	0.0200	7			
A*30	0.0217	12	0.0240	8			
A*31	0.0236	13	0.0360	13			
A*32	0.0354	20	0.0180	6			
A*68	0.0944	53	0.0610	21			
A*34	0.0019	1	0.0000	0			
A*66	0.0019	1	0.0000	0			
B*07	0.1279	72	0.1350	5			
B*08	0.1003	57	0.1350	5			
B*13	0.0295	17	0.0260	9			
B*14	0.0236	13	0.0170	6			
B*15	0.0709	40	0.0740	26			
B*18	0.0531	30	0.0230	8			
B*27	0.0492	28	0.0660	23			
B*35	0.0964	55	0.0700	24			
B*37	0.0177	10	0.0090	3			
B*38	0.0413	23	0.0230	8			
B*39	0.0157	9	0.0300	10			
B*40	0.1377	78	0.0860	30			
B*41	0.0059	3	0.0170	6			
B*44	0.1102	62	0.0920	32			
B*45	0.0059	3	0.0030	1			
B*47	0.0019	1	0.0000	0			
B*48	0.0000	0	0.0030	1			
B*49	0.0236	13	0.0120	4			
B*50	0.0059	3	0.0090	3			
B*51	0.0905	51	0.0530	18			
B*52	0.0118	7	0.0060	2			
B*53	0.0098	6	0.0000	0			
B*54	0.0000	0	0.0030	1			
B*55	0.0059	3	0.0330	12	0.002	0.056	0.15
B*56	0.0078	4	0.0120	4			
B*57	0.0413	23	0.0260	9			
B*58	0.0019	1	0.0140	5			
B*59	0.0000	0	0.0030	1			
C*01	0.0472	27	0.0560	19			
C*02	**0.0472**	**27**	**0.1030**	**36**	**0.002**	**0.026**	**0.43**
C*03	0.1063	60	0.1570	55			
C*04	0.1083	61	0.0990	34			
C*05	0.0709	40	0.0640	22			

Table 1 continued

Allele	Patients ($n = 283$)		Controls ($n = 174$)		p	Pc*	OR
	Frequency	Number of alleles	Frequency	Number of alleles			
C*06	0.0965	54	0.0620	21			
C*07	0.3229	183	0.3320	115			
C*08	0.0157	9	0.0210	7			
C*12	0.0728	41	0.0490	17			
C*14	**0.0492**	**28**	**0.0090**	**3**	**0.002**	**0.026**	**5.99**
C*15	0.0236	13	0.0180	6			
C*16	0.0315	18	0.0060	2	0.017	0.221	5.68
C*17	0.0000	0	0.0090	3			

In bold: statistically significant results

The allele frequencies of healthy controls were taken from http://www.allelefrequencies.net database. Ethnic origin: Caucasoid, Geographic Region: Germany Essen, Source of Population: necro kidney donors and blood donors (Terasaki and Gjertson 1997)

* Pc: Bonferroni corrected p value

Table 2 KIR genotype distribution in patients with different AJCC stages and patients with slow and rapid tumor progression in number and percentages

KIR genotype	Rapid progression (up to 18 months) ($n = 20$)		Slow progression (over 18 months) ($n = 96$)		AJCC I/II ($n = 182$)		AJCC III/IV ($n = 83$)	
	n	%	n	%	n	%	n	%
AA	9	45.0*	23	24.0*	59	32.2	19	22.9
AB	11	55.0	68	70.8	115	63.3	51	61.4
BB	0	0.0	5	5.2	8	4.5**	13	15.7**

* $p = 0.055$, OR = 2.6

** $p = 0.004$, OR = 0.25

specificity. The quantitation of HLA A3/11 in overall patients was given in Table 1. No statistical difference was found when the comparison was estimated in clinical groups defined according to tumor type, progression, ACJJ staging and survival rate.

KIR/HLA Combinations

Since the NK receptor/HLA ligand pairs influence NK-cell function, we evaluated their distribution in melanoma patients. The analysis of the KIR/HLA correlation was possible in 265 melanoma patients. We examined the KIR2DL1 and 2DS1 receptors having HLA-C alleles with Lys motif as their ligands; the receptors KIR2DL2, 2DL3, and 2DS2 and their respective HLA-C alleles with Asn epitope as their ligands; the KIR3DL1 and Bw4 epitope as a ligand (Moretta et al. 1996); the KIR3DL2 and HLA-A*03/A*11 as pairs. The results revealed increased frequency of 2DL2 in the presence of C1 ligand in patients with AJCC III and IV stages in comparison to individuals with AJCC I and II stages. The difference was significant between individuals with AJCC I and AJCC III stage ($p = 0.038$; OR = 2.20) and between patients with AJCC I and subjects with stage III and IV grouped together (metastatic disease) ($p = 0.045$; OR = 1.93; Table 4). No significant difference was determined with respect to the KIR/ligand combinations including KIR-Bw4 and KIR-HLAA*03/*11 associations and the remaining parameters of disease type and progression.

Discussion

Numerous studies have shown that melanoma is a highly immunogenic tumor. A number of mechanisms enabling tumor cells to escape destruction by the immune system

Table 3 KIR gene distribution in patients with rapid and slow progression of the disease

KIR gene	Patients ($n = 20$) with rapid melanoma progression (up to 18 months) (%)	Patients ($n = 96$) with slow melanoma progression (over 18 months) (%)
2DL1/2DP1	100.0	100
2DL2	45.0	57.3
2DL3	100.0	93.8
2DS1	30.0	38.5
2DS2	45.0	57.3
2DS3	20.0	32.3
2DS4	100.0	92.7
2DS5	20.0	35.4
3DL1	100.0	95.8
2DL5A*001/005	30.0	50.0
2DL5B*002/004	20.0	26.6
2DL5B*003	0.0	1.1
2DS4*001/002	35.0	36.4
2DS4*003/006	85.0	88.5
3DP1*001/002/004	35.0	34.4
3DP1*003	65.0.0	65.6

have been found in advanced melanoma lesions. Such mechanisms are the loss of expression of HLA class I molecules, loss of or mutations in tumor-specific antigens, secretion of inhibitory factors including cytokines like IL-10 and TGF-β, promotion of vascular development, etc. Apart from a well described alteration of class I peptides in tumors (Garrido et al. 2010), the role of genetic diversity of HLA class I and II molecules in the immune escape mechanisms in MM can only be speculated. Key to the immune response are the HLA class II proteins—a variety of studies have been conducted attempting to link these molecules to the risk of developing melanoma. All these studies, however, produced inconsistent or controversial results (Bonamigo et al. 2012). Data on HLA class I correlation with the disease (Bettinotti et al. 2000) are scarce, and no consensus has been reached yet regarding the association of specific class I loci with the susceptibility to melanoma. Our previous study among Bulgarian population showed slightly increased frequencies of HLA-A*01, B*08, and Cw*01 alleles in melanoma patients (Naumova et al. 2005) and only the class I and Class II combined haplotype (A*01-B*35-Cw*04; A*01-B*08-DRB1*03 and A*24-B*40-DRB1*11) analysis conferred a predisposition to MM. Metzelaar-Blok et al. (2005) reported that HLA class I and class II polymorphism represented no risk with respect to the development of melanoma with uveal location. Our study covered a large number of subjects demonstrating a predisposition effect of HLA-C*14 allele and a protective effect of HLA-C*02 allele on disease development among German patients, the analysis being performed in the disease heterogeneous MM group. For a long time HLA-C loci have been neglected to some extent, due to their relatively low level of cell-surface expression and apparently insignificant role in mediating antigen-specific T cell responses. However, it has been accepted undoubtedly that the major role of HLA-C is to act as a ligand for KIRs. HLA-C alleles, usually along with their corresponding KIRs, have been regarded as protecting against or resulting in susceptibility to several malignancies (Martin et al. 2010). The data contained in this study support the involvement of the HLA-C locus in modulating the risk of MM, as this is probably either due to or the result of differential KIR/HLA-C mediated interactions. Unfortunately, however, it was impossible to prove this statement in practice owing to lack of data on the KIR/HLA combinations in healthy German individuals, used for the HLA association analysis.

The KIR gene frequencies observed in patients were very similar to those found in Germans and other Caucasian populations (Norman et al. 2001). A difference in the KIRs, however, was established between the rapidly

Table 4 KIR 2DL2/C1 distribution in patients with MM staged according to AJCC classification

KIR/ligand	All patients ($n = 265$)	%	AJCC I ($n = 71$)	%	AJCC I/II ($n = 24$)	%	AJCC II ($n = 87$)	%	AJCC III ($n = 47$)	%	AJCC IV ($n = 36$)	%
$2DL2^+/C1^+$	123	46.4	27	38.0*	10	41.7	41	47.1	27	57.4*	18	50
$2DL2^+/C1^+$			27	38.0**					AJCC III + AJCC IV			
									$n = 45$ (54.2%)**			
$2DL2^+/C1^-$	17	6.4	3	4.2	3	12.5	8	9.1	1	2.1	2	5.5
$2DL2^-/C1^+$	101	38.1	34	47.9	9	37.5	32	36.8	15	31.9	11	30.5
$2DL2^-/C1^-$	24	9.1	7	9.9	2	8.3	6	4.0	4	8.5	5	13.9

* $p = 0.038$, OR = 2.20

** $p = 0.045$, OR = 1.93

and slowly progressive groups, based on the lower frequency of activating 2DS5 in cases of rapidly progressing cancer. The recognition of HLA-C molecules by KIR2DS5 was shown to be successful only in the presence of certain peptides. The main mechanism of action of this receptor is to induce cytokine release and cytotoxicity by means of the DAP12 signaling polypeptide (Della Chiesa et al. 2008). Therefore, a speculation is possible that the over-representation of KIR2DS5 in patients characterized by a slow progression of the disease could prove beneficial for the genetically determined enhanced cytotoxic capacity of NK cells against tumor cells. It is widely accepted that two different KIR genotypes A and B play different and nonpareil roles in the biology of NK cells. The data we accumulated showed that the AB genotype was prevalent among MM patients, followed by AA and BB genotypes (61.4, 29.2 and 9.2%, respectively). These numbers correspond to the KIR genotype frequencies determined in a variety of healthy Caucasian populations (Toneva et al. 2001). The comparison between clinically defined groups, however, showed that having the AA genotype (prevalence of inhibitory signals) was an unfavorable marker for disease evolution, since higher values of this variant were found in patients with rapid progression disease (borderline significance; Table 2); on the other hand, the prevalence of BB profiles in the genome of patients with metastases (AJCC III/IV) was statistically significant ($p = 0.004$). These data can be difficult to interpret when disease modulating effect in an inhibitory manner is presupposed. However, recently described NK cell licensing phenomenon can help to understand these findings (Kim et al. 2005). The authors postulated that NK cells show evidence of enhanced proliferation if they express an inhibitory receptor for MHC class I molecules. It seems that the inhibitory receptors with immunoreceptor tyrosine-based inhibitory motif in the cytoplasmic domain are responsible for the signals that direct NK cell licensing. This process results in generation of licensed self-tolerant NK cells or unlicensed ones. The prevalence of genotypes with low gene content for inhibitory KIRs in patients with metastasis may interfere with self-tolerance mechanisms implacable in tumor cells recognition.

The function of NK cells largely depends on the inheritance of both HLA-C/Bw4 and KIR molecules. Both inhibitory and activating receptors, recognizing HLA-class I molecules on target cells, regulate the NK cell activity (Khakoo and Carrington 2006; Ljunggren and Karre 1990), thus influencing the ability of NK cells to kill tumor-derived cells or cells exposed to stress. Therefore, effector functions are only observed when the inhibitory signals are overwhelmed by the activating ones. A variety of receptor/ligand combination profiles can be observed in different individuals, due to the high polymorphism of KIR and HLA genes and their independent segregation (Nelson et al. 2004). In this way such HLA/KIR combinations can cause differences in the respective levels of NK cell activation, which may have a strong effect on disease susceptibility or severity (Rajagopalan and Long 2005). A number of previous studies have revealed the involvement of KIR receptor/ligand pairs in the pathogenesis and progression of different human diseases, including cancer (Middleton et al. 2007; Mihaylova et al. 2008). Campillo et al. (2013) demonstrated that KIR2DL3(−)/C1C2 genotype correlated with the nodular type of melanoma and tumor ulceration and the KIR2DL1(+)/S1(−)/C2C2 profile was associated with susceptibility to melanoma and sentinel lymph node metastasis formation. In the present study, we did not identify any significant influence of the above-mentioned KIR/ligand pairs on the patients' groups analyzed. A possible cause could be a different study design and different groups of comparison. It is well known that KIR2DL1 provides stronger inhibition, compared to KIR2DL2 or KIR2DL3. However, Schönberg et al. (2011) have demonstrated recently the dominant suppressive effect of KIR2DL2 presence. According to the authors, the developing NK cells trigger the expression of KIR2DL2 but do not favor further inhibitory receptor involvement. Therefore, our study linked the increased frequency of KIR2DL2 observed in the presence of C1 ligand in AJCC III and IV groups to a strong inhibition. Such an increased frequency may also be relevant to the development of metastases in melanoma patients. The increased understanding of the intricacy of KIR biology and KIR/ligand interactions has provided recent opportunities to use NK cells as an immunotherapeutic tool for cancer beneficial influence. Data presented here could be used as valuable predictive markers and as a means of selecting patients for HLA restricted immunotherapy. In support of the benefits of similar therapeutic strategies, Schadendorf et al. (2006) have shown that the individual HLA-haplotype pattern could have a major impact on vaccination efficacy in melanoma metastatic patients, treated with autologous peptide-pulsed dendritic cells. Identification of a predictive marker like HLA-Cw06 in patients with MM also allowed selecting individuals who responded to adjuvant interferon treatment with better relapse-free and overall survival (Gogas et al. 2010).

In conclusion, alterations to the NK cell receptors/ligands interactions which seem to be genetically predetermined may result in imperfect recognition of neoplastic cells. In this study we have addressed the relevance of KIR/ligand gene polymorphism to the evolution and prognosis of MM. Although it is possible that there is no direct association between the KIR gene content/HLA ligands and the risk of MM development, we have demonstrated that once the malignancy has occurred,

particular KIR genes, genotype profiles and KIR/ligand combinations confer a disease modulating properties consequently to NK cell licensing effect from multiple inhibitory KIRs and their cognate HLA ligands. In general, the results presented here may help in assessing disease progression and deserve further investigation.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest with regard to the work presented in this article.

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