ORIGINAL ARTICLE

Expression of Killer Cell Immunoglobulin-like Receptors (KIR) in Sex-associated Malignancies

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ABSTRACT

Introduction: Sex shapes immune response with possible consequence on tumor immune escape. Acute lymphoblastic leukemia (ALL) predominates in males while ovarian cancer (OC) occurs in females. NK cells essential for tumor killing may have male preponderance. Association of sex, NK cell activity and malignancies is unclear. We hypothesize that sex differentially affects KIR expressions in sex-biased cancers. **Method:** Expression of inhibitory (*KIR2DL1-5* and *KIR3DL1-3*) and activating (*KIR2DS1-2* and *4-5* and *KIR3DS1*) genes in B-, T-cell ALL, OC and normal controls were determined by reverse-transcription polymerase-chain-reaction. **Result:** All normal males (but not females) expressed the framework genes and generally maintained haplotype A, except *KIR3DL1*. Normal females expressed more activating KIRs. Frequencies of *KIR2DL1*, *2DL4* and *2DS2* were significantly reduced among ovarian cancer patients. Sex difference in frequencies of KIR expression was not detected in ALL as majority were undetectable except framework gene *KIR3DL2*, was more frequent among T-ALL. **Conclusion:** Cancers may be associated with reduced KIR expression and influence of sex requires investigation.
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Keywords: Sex, NK cells, Killer-cell immunoglobulin-like receptors (KIR), Acute lymphoblastic leukemia (ALL), Ovarian cancer (OC)

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INTRODUCTION

Many cancers affect more males than females (1) including hematological malignancies such as acute lymphoblastic leukemia (ALL). ALL affects immature lymphoblasts in bone marrow (BM). Majority of ALL are B cell lineage, with 15% of T-cell origin (2). ALL is reported to predominate among males, from 1.2 times in B-cell lineage to 2.2 in T-cell ALL (3). In the past 20 years, immunotherapeutic strategy has been used for the treatment of leukemia such as in CAR-T cell therapy.

Ovarian cancer arises in the female reproductive organs and is one of the leading causes of malignancies and cancer-related deaths in women (4). Due to paucity of symptoms, most cases of ovarian cancer are diagnosed at an advanced stage. Current treatment modality for ovarian cancer involve surgery and use of adjuvant-chemotherapy techniques, however, these are not fully effective with most patients relapsing (5). Immunotherapy using natural killer (NK) cells is currently explored to complement or even replace chemotherapy in ovarian cancer patients (6).

NK cells are important in removing abnormal cells to prevent tumor formation. The mechanism of NK cell activation is known as the "missing-self" hypothesis where, in contrast to T cells, in the absence of human leukocyte antigen (HLA) class I expression, NK cells are activated. Tumor cells may down-regulate expression of HLA-class I resulted from mutation. This "non-self"

circumstance due to the lack of self-antigen expressions (7) is an early innate response to kill tumor cells without prior sensitisation and a reason for the increased interest in developing NK cells in adoptive cell therapy against cancers (8). This is also a viable alternative to cure otherwise fatal leukemia in patients with no HLA compatible donors.

In normal cells (9) interaction between HLA-C and HLA-G (as ligands) and inhibitory killer-cell immunoglobulin-like receptors (KIRs) i.e. *KIR2DL1-5* and *3DL1-3* triggers an inhibitory signal to stop NK cells from killing normal cells. This represents a true checkpoint in regulation of NK cell function. Other ligands of KIRs are still unknown (10).

Activating receptors of NK cells are the *KIR2DS1-5* and *KIR3DS1*, C-type lectin like receptors and leukocyte inhibitory receptors (LIR) (11). Inhibitory KIR receptors are effected by the long (L) cytoplasmic tails, attached to inhibitory immunoreceptor tyrosine-based inhibitory motif (ITIM); while short (S) cytoplasmic tails are attached to activating immunoreceptor tyrosine-based activation motif (ITAM) (7).

The KIR gene family located in chromosome 19 is made up of 15 genes including 2 pseudogenes and similar to HLA, is highly polymorphic (12). KIR polymorphism arises from inheritance of different sets of KIR genes, allelic genes variation as well as functional capability of expressed protein (13). The KIR haplotype exhibits extensive variation in the number and type of KIR carried. Nevertheless, each haplotype has four common framework genes, organised with *KIR3DL3* at the centromeric end, *KIR3DL2* at the telomeric end and *KIR3DP1* and *KIR2DL4* in the middle of KIR gene cluster. There are two basic haplotypes termed haplotypes A and B with genotypes (AA and Bx, where x can be A or B). Haplotype A is inhibitory and composed of an additional three inhibitory genes (*KIR2DL1*, *2DL3* and *3DL1*) and one activating gene (*KIR2DS4*). Haplotype B is activatory and is more variable and combines with one or more of the following genes, *KIR2DL2, KIR2DL5A/B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5* and *KIR3DS1* genes. Over 40 distinct group B have been defined in IPD database (https://www.ebi.ac.uk/ipd/).

Down-regulation of activating NK receptor expression is an important mechanism of tumor escape, allowing these cells to elude NK-mediated control of tumor growth and metastatic spread. Individuals expressing more than five activating KIR genes are found to be more susceptible to the risk of nasopharyngeal carcinoma (14). Altered expressions of NK cell activation receptors have also been reported in breast cancer patients (15). In ovarian cancer patients NK cell activity was significantly lower in patients whose disease progressed than in patients with progression-free survival (16) suggesting critical role of NK cell in this cancer.

Extensive review has demonstrated that male and female differ in immune responses (17,18). Sexual dimorphism in anti-metastatic control by NK cells in mice is found to be significant (19). Literature has also shown that NK cells predominate in male (20–23). An 11-year follow up of 3625 Japanese residents for cancer incidence found the cytotoxic activity of PB lymphocytes in age-adjusted relative risk of cancer incidence was lower among females (24). Evidence on role of sex on NK cells activity in tumor development is few and requires further investigation. In this study, we used ALL, which affecting both male and female, as well as OC in woman as medium to understand the significance of NK cell KIR expression in both sexes. We hypothesize expression of KIR genes involved in regulation of NK activity are affected in tumors in a sex specific manner.

MATERIALS AND METHODS

Bone marrow samples of acute lymphoblastic leukemia

Bone marrow (BM) samples in EDTA tubes (BD Biosciences, US) were collected from ALL patients admitted in Hospital Kuala Lumpur (HKL). Seven B-ALL male, 7 B-ALL female and 7 T-ALL male subjects were included in the study.

Peripheral blood samples of ovarian cancers and normal controls

EDTA-anticoagulated peripheral blood (PB) was collected from 13 ovarian cancer patients diagnosed in HKL.

PB samples provided by apparently healthy volunteers (with no acute or chronic disease including malignancies, not pregnant or on any immunomodulation therapy/ procedure) were used as normal controls. Eight normal male and 9 normal female controls were recruited.

The study was approved by the Institutional Medical Research Ethics Committee, Universiti Putra Malaysia and Medical Research and Ethics Committee, Ministry of Health Malaysia and informed, written consents were obtained from all subjects.

Isolation of mononuclear cells

Mononuclear cells (MC) from healthy volunteers, ALL and ovarian cancer patients' sample were isolated from PB/BM samples using density gradient centrifugation on Ficoll-Paque Plus (GE Healthcare, Sweden).

Flow cytometry

Half a million mononuclear cells (MC) isolated from BM samples were tagged with CD45-PerCP (BD Biosciences, USA) using standard surface marker staining protocol. At least 10,000 cells were acquired on FACSCalibur and analyzed for percentage of CD45+ lymphocytes with Cell-QuestPro software (both of Becton Dickinson, USA).

RNA extraction, DNase treatment, reverse transcription, and cDNA synthesis

RNA was extracted from the isolated MC according to manufacturer's instructions (Tri-Reagent; Molecular Research, US). Extracted RNA was treated with 2000 U/ml DNase I (New England Biolabs, UK) and 40 U/ml recombinant RNasin ribonuclease inhibitor (Promega Corporation, US). Reverse transcription and cDNA synthesis were performed on 4000 ng RNA with oligo dT primers (Promega Corporation, US), M-MLV reverse transcriptase, RNase inhibitor (Promega Corporation, US), and dNTP (Fermentas, US) according to manufacturer's instructions. Converted cDNA was kept in -30°C for further analysis.

Polymerase chain reaction and KIR primers

Duplex polymerase chain reaction (PCR) was performed to amplify inhibitory KIR genes: *2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2* and *3DL3*, activating KIR genes: *2DS1, 2DS2, 2DS4, 2DS5* and *3DS1*, while beta-actin (Actin) gene was used as internal reference control. All KIR primers used in this study were previously described (25–27). The RT-PCR method was designed to detect transcripts that were KIR gene specific, but insensitive to allelic variations in those genes (26). Primer sequences are shown in Table I. Briefly, PCR reaction consisted of 3 mM of $MgCl_2$, 0.2 mM dNTP, 5U Taq polymerase (Fermentas, US), 0.06 µM each of Actin forward and reverse primers, 0.5 µM each of respective KIR forward and reverse primers and 1 µl cDNA. PCR profile: initial denaturation at 95°C for 2 min, then 4 cycles of 60 s at 95°C, 60 s at 61°C and 45 s at 72°C; 28 cycles of 30 s at 95°C, 45 s at 60°C, 45 s at 72°C, and finally 10 min at 75°C. PCR products were separated on 2% agarose gel and viewed using FluorChem 5500 software.

Statistical analysis

Fisher's Exact Test compared absent/present of KIR gene expression between two group. Statistical analysis was performed using SPSS (version 21.0) and GraphPad Prism 8.0. $p<0.05$ was considered significant.

RESULTS

Clinical features

Clinical features of B-ALL/T-ALL and ovarian cancer patients are described in Table II. Diagnosis was determined by haematologists and histopathologists, respectively. Acute leukemia subjects were not matched for age in healthy volunteers as only >18 years old were included in the study.

CD45+ lymphocyte

The percentage of lymphocytes in BM samples was determined from the population of cells with low side scatter and low forward scatter which express high levels of CD45 (Fig. 1). This is standard strategy to differentiate lymphocytes from lymphoblasts in acute leukemia samples. Average (±standard deviation) lymphocyte percentage in female B-ALL, male B-ALL and male T-ALL BM were 4.2 (±3.08), 5.40 (±6.34) and 2.15 (±1.09), respectively.

KIR gene expression

Since the populations of NK cells may vary between sites of sampling, only those from the same sites i.e. PB from normal and ovarian cancer patients and BM samples from ALL patients, were compared.

Fig. 2 shows gel electrophoresis of PCR amplified bands following reverse-transcription polymerase chain reaction for KIR gene *KIR2DL2* and beta-actin as internal control. Data collected was present or absent of the KIR band when the beta-actin band is present. Not all samples were successfully amplified in the female group. The following are the actual numbers of normal female samples used in subsequent analysis:

Table I: Sequences of KIR primers

KIR Genes	Forward	Reverse	Product sizes (bp)
Actin	CGC GAG AAG ATG ACC CAG ATC[1]	TTG CTG ATC CAC ATC TGC TGG[1]	734
2DL1	TCT CCA TCA GTC GCA TGA C[2]	CAG AAT GTG CAG GTG TCG[2]	488
2DL2	GCA ATG TTG GTC AGA TGT CAG[2]	GCC CTG CAG AGA ACC TAC A[3]	383
2DL3	CCA CTG AAC CAA GCT CCG[1]	CAG GAG ACA ACT TTG GAT CA[1,3]	353
2DL4	AAC ATC TTC ACG CTG TAC AAG A[2]	GCC TGG AAT GTT CCA TTG AT[2]	368
2DL5	TGG TCA CAG GTC TAT TTG G[2]	GGT TCA GTG GGT GAA GAT G[2]	340
3DL1	ACA TCG TGG TCA CAG GTC C[1]	ACA ACT TTG GAT CTG GGC TT[2]	633
3DL2	CGG TCC CTT GAT GCC TGT[1,3]	GAC CAC ACG CAG GGC AG[1,3]	369
3DL3	CAC GAT GCG GGT TCC C[2]	AGA AGA GGA GGA TAG CAA AGG[2]	508
2DS1	TCT CCA TCA GTC GCA TGA A[1,3]	AGG GCC CAG AGG AAA GTT[1,3]	314
2DS2	TGC ACA GAG AGG GGA AGT A[1,3]	CAC GCT CTC TCC TGC CAA[1,3]	257
2DS4	GGT TCA GGC AGG AGA GAA T[2]	TTG ACC ACT CGT AGG GAG C[2]	218/196
2DS5	AGA GAG GGG ACG TTT AAC C[1,3]	TCC GTG GGT GGC AGG GT[2]	396
3DS1	GGC ACC CAG CAA CCC CA[1]	AAG GGC ACG CAT CAT GGA[1]	247

[1]These primers were previously described by Uhrberg, et al (1997) (39)
[2]These primers were previously described by Thompson, et al (2006) (26)
[3]These primers were previously described by Gymez-Lozano & Vilches (2002) (27)

Table II: Characteristics of ALL patients, ovarian cancer patients and normal controls

	B-ALL (n=16)	T-ALL (n=8)	Normal control (n=16)	Ovarian cancer (n=13)
Sex				
Male	8	8	8	0
Female	8	0	10	13
Age				
Average year (range)	26.3 (7-62)	31.4 (11-75)	33.1 (24-50)	50 (20-70)
Lab Diagnosis				
Average lymphoid blast % (range)	59.0 (12.4-86.2)	82.9 (63.0-94.7)	-	Histo-pathological diagnosis
Average lymphocyte % (range)	6.13 (0.8-22.4)	2.3 (1.2-4.5)		
Cytochemistry	Periodic Acid Schiff (PAS)$^+$ and Myeloperoxidase$^-$			
Immunophenotyping	CD19$^+$,CD10$^+$, CD10$^+$, CD20$^+$, CD22$^+$, CD79a$^+$, CD34$^{+/-}$, HLA-DR$^+$	cCD3$^+$, CD7$^+$, CD2$^+$, CD5$^+$, CD8$^+$, CD1a$^{+/-}$, HLA-DR$^{+/-}$		

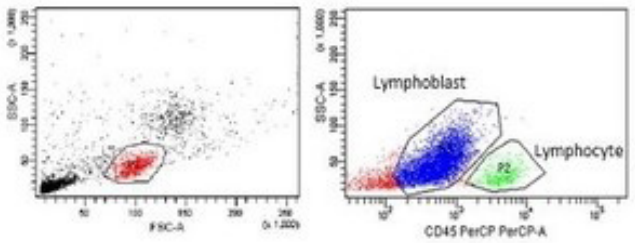

Figure 1: Representative flow cytometry plots showing sequential gating on mononuclear cells with low side scatter and low forward scatter (left panel) and high intensity CD45 cells to identify lymphocyte population (right panel).

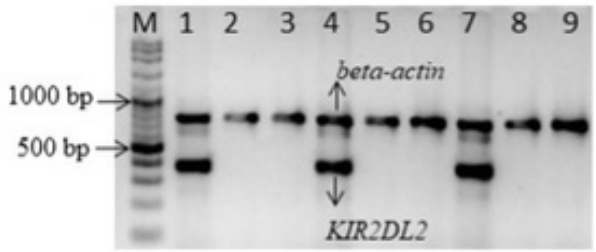

Figure 2: Representative gel electrophoresis of KIR2DL2 and beta-actin (internal control) following reverse transcription-polymerase chain reaction (rt-PCR) of RNA samples (1-9). Sample 1 to 5 are the normal controls while 6 to 9 are the ALL samples. Upper band is the PCR product of beta-actin (734 bp) while the lower band is the PCR product of KIR2DL2 (383 bp). Absent of lower band indicates *KIR2DL2* was not expressed in that sample. M is 100 bp DNA ladder

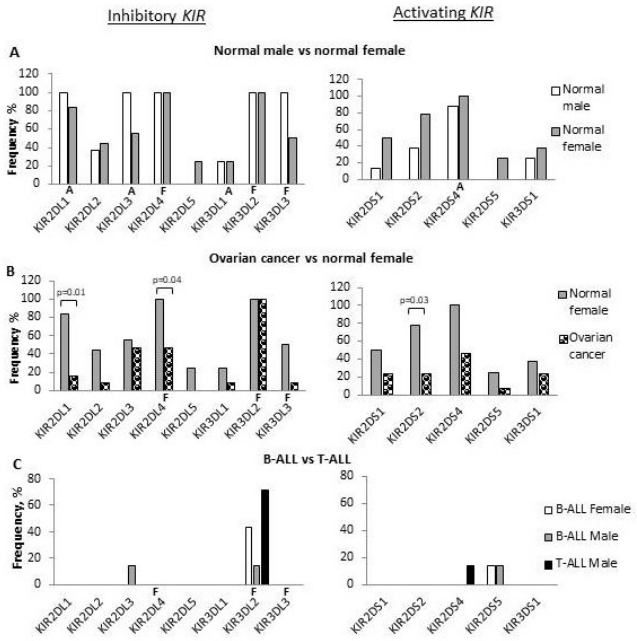

Figure 3: Bar chart compares frequency of activating and inhibitory KIR genes present in samples. A) normal male, all KIR (N=8) versus normal female, KIR2DL2, 2DL3, 3DL2, 2DS2 (N=9), KIR2DL5, 2DS5, 3DS1 (N=8), KIR2DL4 (N=7), KIR2DL1 (N=6), KIR2DS4 (N=5), KIR3DL1, KIR3DL3 (N=4). B) ovarian cancer female, all KIR (N=13) vs normal female (same as A). C) B-ALL female, all KIR (N=7) vs B-ALL male, all KIR (N=7) vs T-ALL male, all KIR (N=7). Peripheral blood samples were collected from normal and ovarian cancer patients. Bone marrow was collected from ALL patients. F – framework genes. A – haplotype A. * indicates statistical significance p<0.05.

KIR2DL2, 2DL3, 3DL2, 2DS2 (N=9), *KIR2DL5, 2DS*5, *3DS1* (N=8), *KIR2DL4* (N=7), *KIR2DL1* (N=6), *KIR2DS4* (N=5), *KIR3DL1, KIR3DL3* (N=4).

Among normal male controls, 100% expressed the investigated framework KIR genes, *2DL4, 3DL2, 3DL3* as well as two other inhibitory genes (*KIR2DL1* and *KIR2DL3*). In contrast, only two framework genes (*KIR2DL4 and KIR3DL2*) were expressed by all normal females. Furthermore, frequency of the third framework gene (*3DL3*) and another inhibitory gene (*KIR2DL3*) were expressed at lower frequency than male, though none reached significance (p=0.08 and p=0.09, respectively). The frequencies of activating KIR genes, on the other hand, were higher among females in all five activating KIR genes tested, though none were significantly different (Fig. 3A).

In general, frequency of ovarian cancers expressing inhibitory or activating KIRs were lower compared to

normal females with significantly lower frequencies in *KIR2DL1* (p=0.01), *KIR2DL4* (p=0.04) and *KIR2DS2* (p=0.03). Expression of framework gene *KIR3DL2* was maintained in all ovarian cancer patients (Fig. 3B).

Among ALL cases, comparisons were made between sex groups of B-ALL and T-ALL as no normal BM samples were obtained. As per normal and ovarian cancers, majority of T-ALL male maintained expression of *KIR3DL2*, one of the framework genes. Interestingly, expression of this gene this was lost among B-ALL samples. No significant difference was observed between male and female B-ALL. Expression of other KIR genes were almost undetectable in ALL (Fig. 3C).

DISCUSSION

DNA genotyping demonstrates there is high variability in KIR receptors. Uhrberg et al. (1997) determined that all individuals (N=10) express (phenotype) the KIR genes that they possess (genotype). Here, *KIR2DL1*, framework genes *KIR2DL4* and *3DL2* and *KIR2DS4* were the most frequently expressed (>80%) in healthy individuals. This supported earlier studies which demonstrated similar patterns in highest frequencies of expression for these four genes among healthy individuals [Uhrberg et al., (1997) (N=52) (25); Chen et al., (2009) (N=32) (28)] (*3DL3* and *KIR3DP1* were not examined in those studies). Among activatory KIRs, again consistent with the earlier studies, there was variation in expression among healthy subjects which is consistent with inheritance of the genotype. Only *2DS4*, of haplotype A, was present in >80% individual, consistent with the other two studies. Subsequent studies suggest that allelic variation has impact on KIR protein expression and subsequently function (12). It was reported, of the four conserved KIR genes only *KIR2DL4* is expressed at both RNA and protein level in all individuals. This receptor is unique as it has both inhibitory and activating KIR features. Protein analysis confirmed that expression of *KIR2DL4* was restricted to NK but not T, B or monocytic cell lines and only to CD56+ but not CD3+CD56- cells in PB. Importance of this receptor is indicated by the expression of its specific ligand, HLA-G on fetal trophoblast cells, and thus in regulation of maternal decidual NK cells in protection of fetal cells (29). Lack of *3DL3* cell surface expression in peripheral blood mononuclear cell (PBMC) is suggested to be due to regulation via miRNA (13). Similarly, we observed expression of *3DL3* at the mRNA level.

Comparing frequencies of expression between sexes, none of the KIR was significantly different. Male showed tendency to express more inhibitory KIRs with many, particularly of haplotype A (addition of *KIR2DL1, 2DL3, 3DL1* and *KIR2DS4* to framework genes) expressed in all 100%. This is also the pattern observed in general (25,28). The exception being *KIR3DL1* which was infrequently present among our samples. This difference with the other studies is unclear. Lesser females demonstrated haplotype A as frequencies for KIR2DL3 and *3DL3* were lower than 100%, unlike male. In contrast, frequencies of activating KIR genes were higher among females. Very few other reports compared between sexes. Trundley et al., (2006) reported higher and more frequent *3DL3* mRNA in PBMC of healthy females (N=15), in contradiction to ours. However, levels, in general, were low and protein expression was not detected. Nevertheless, protein expression might be induced under certain developmental or pathological situations (30). It is possible that external stimulation may alter expressions to become significantly different between genders as observed by in vitro PHA stimulation of PB cells (31).

Chen et al. (2009) noted sex hormones may not affect KIR expression as no difference in expression between male and female was observed. However, high expression of estrogen receptor-alpha (ERa)/Esr-1 gene is observed on NK cells suggesting NK cells may be potential targets for estrogen which were shown to reduce NK cell cytotoxicity against tumor cell line [reviewed in Blanquart et al., 2021 (32)]. This may have a profound sex-biased effect on tumor immune escape. On the other hand, frequencies of KIRs expressions were significantly lower in older age group (28).

In general, a loss in KIR gene expressions was observed in cancer patients. No complete loss of KIR expressions was observed, indicating that NK cells were still present in ovarian cancer samples. Frequencies of two inhibitory (*KIR2DL1*and *2DL4*) and one activating gene (*KIR2DS2*) were significantly reduced in ovarian cancers compared to healthy females. In theory, absence of inhibitory KIR implied loss in self-inhibition in NK cells and ability to kill. A study on breast cancer also observed lower frequency of the *2DL1* genotype compared to healthy controls suggesting a genetic risk for individuals lacking *2DL1* (33). The absence of *KIR2DL4*, a framework gene which was present in all healthy samples was interesting as it suggested a suppressive action rather than a variation in genotype. Lin et al. (2007) detected expression of HLA-G, ligand for *KIRDL4*, in 66.7% primary ovarian carcinoma which was absent in normal ovarian tissues (34). Furthermore, Rutten et al. (2014) showed expression of HLA-G (but not HLA-A, B/C or E) independently predicted improved survival in ovarian cancers. KIR ligand-receptor binding induces secretion of cytokines and chemokines (35). Cytokines such as IL-12/15/18 were shown to downregulate surface expression of *KIR2DL2/L3*, *KIR2DL1* and *KIR3DL1* receptors on peripheral blood NK cells and subsequently enhanced cytotoxicity of NK cells towards the tumor cells (36). Combining these observations, it appeared tumor immunity by NK cells remained intact in these patients and following possibly debulking with cancer therapy was able to provide long term protection and increased patient survival. On the other hand, *2DL4* is

predicted to have both inhibitory and activating actions. Absence in expression may result in reduced ability to trigger activation of killing in NK cells.

We did not find reports associating 2DS2 with cancers, however, a recent study revealed certain cancer cells express the ligand for *2DS2* and are thus potential targets for NK cell lysis (37). Suppressing the KIR activating receptors may help tumor cells escape immune responses. Tumor cells may alter KIR gene expression through various mechanisms, including release of soluble factors by tumor cells as well as other cells in the microenvironment such as M2 macrophages, myeloid-derived suppressor cells (MDSC), T-reg and stromal cells that are attracted and/or "conditioned" by tumor cells (12). Prostate, breast and ovarian but not in melanoma, colorectal adenocarcinoma, leukemia or esophageal carcinoma cell lines strongly activated responses in cells expressing the *KIR2DS2* receptor (37). This implied a potential role of *2DS2* in sex-associated cancers.

Acute leukemia samples provided an opportunity to compare sex differences in KIR gene expression particularly as there is a higher preponderance among males. BM samples provided a small percentage of lymphocytes, ranging from average 2.15% – 5.40%. Analysis of these samples showed loss in majority of KIRs gene expression. While an average of seven KIR genes were expressed in healthy controls only 0.5 was expressed in each ALL samples. One reason may be due to the low number of lymphocytes in the BM samples, however, two PB samples from acute leukemia samples with lymphocytes at 3.4% to 22.4%, also demonstrated similar loss in expression of KIR genes (data not shown). In contrast to B-ALL, frequency of T-ALL samples that maintained expression of *3DL2* was higher, albeit being the group with lowest percentage of lymphocytes. Detectable gene expression of this framework gene also indicated presence of sufficient NK cells in the sample. Undetectable levels of the other genes suggested that these cells no longer expressed the receptors. In contrast, among B-ALL samples, even expression of *3DL2* was almost absent even though more lymphocytes were detected, implying a stronger effect on NK cells in B-ALL patients.

Examining genotype of activating KIRs in pre-B ALL paediatric patients (N=100) in a Canadian study revealed a significantly lower frequency of *2DS1-5* and *3DS1* compared to controls (N=245). On the other hand, only *2DS1-3* were significantly lower among T-ALL (N=30) [30]. In contrast, none of the genes frequencies were significantly different in a Bulgarian study comparing ALL of an older age group (N=52) and healthy individuals (N=126) (38). The significantly lower frequency in the genotype may explain the low expression of these genes among ALL patients here. There was also no difference of genotype between ALL and normal samples for the KIR inhibitory genes (38).

CONCLUSION

Standardisation of methods may help in providing better comparative support evidence. In summary, all males (but not female) expressed the framework genes and generally maintained haplotype A, except *KIR3DL1*. Females expressed more activating KIRs. Frequencies of *KIR2DL1, 2DL4* and *2DS2* were significantly reduced among ovarian cancer patients. All KIRs were undetectable in ALL except framework gene *KIR3DL2* which was detected more frequent among T-ALL. This preliminary study on sex differences in KIR gene expressions need to be further validated by KIR genotyping and with a larger sample size and the analysis was limited with low number of samples. Nevertheless, NK cells in this study were evaluated on the expression on CD56 alone, without addressing CD3-co-expression, in which may have included the NKT cells. B cells and monocytes also were not discriminated by the expression of CD19 and CD14 respectively.

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