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The impact of *KIR2DS4* gene on clinical outcome after hematopoietic stem cell transplantation

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ABSTRACT

Killer cell immunoglobulin-like receptors (KIR) are a family of inhibitory/activating receptors expressed on NK cells. Interactions of KIR receptors with KIR ligands have been shown to modify hematopoietic stem cell transplantation (HSCT) outcome. The aim of this research was to determine the *KIR2DS4* allele variants distribution among 111 patients with different hematological malignancy who underwent HSCT and their donors, and to evaluate *KIR2DS4* alleles' impact on HSCT outcome. The KIR gene frequency analysis showed a significantly higher incidence of full-length *KIR2DS4* alleles among patients. The impact of *KIR2DS4* alleles on transplantation outcomes revealed that donors' full-length *KIR2DS4* alleles is associated with lower overall survival rates, higher risk of GVHD and higher relapse incidence. The expression of full-length *KIR2DS4* allele variants may contribute to a worse clinical outcome after HSCT. KIR typing for *KIR2DS4* could be used as an additional criterion for selecting suitable donors in cases when more than one HLA identical donor is identified for a specific patient.

1. Introduction

Killer cell immunoglobulin-like receptors (KIRs) are genetically very polymorphic receptors exerting inhibitory or activating functions after interaction with their ligands, and thus regulating the activity of natural killer (NK) cells [1]. The biology of inhibitory KIRs is well defined in contrast to the elusive physiological role of activating KIRs. The main ligands for the majority of KIR receptors are human leukocyte antigen (HLA) C molecules classified into subgroups C1 and C2 according to their residue on position 80 (HLA-C group 1, asparagine (N) at position 80: C*01/03/07/08/12/13/ 14/15:07/16:01; HLA-C group 2, lysine (K) at position 80: C*02/04/05/06/12:42/15/16:02/17) [1,2]. Other known ligands include a subset of HLA-A (HLA-A*23/24/32) and HLA-B (HLA-B*15:02/27/37/38/44/47/49/51/52/53/57/58/59) molecules that carry a Bw4 epitope [3]. However, the ligand specificity of activating KIRs is less well-described, although possible candidate ligands might be some non-HLA proteins such as tumor-specific antigens or foreign antigens expressed on infected cells [4]. All KIR genes can be classified in two general haplotype groups: haplotype A, with *KIR2DS4* as the only activating KIR gene and haplotype B, containing at least one activating KIR gene other than *KIR2DS4*.

KIR2DS4 is the most prevalent, evolutionarily oldest, and most divergent activating KIR receptor. A unique ligand specificity was proven for the *KIR2DS4* receptor which interacts with a subset of HLA-C group 1 (HLA-C*01:02, C*14:02, C*16:01) and HLA-C group 2 (HLA-C*02:02, C*04:01, C*05:01) molecules as well as with HLA-A*11:01 and A*11:02 molecules although binding to A*11:02 is four times higher than to A*11:01 [5]. *KIR2DS4* is the only activating gene within the KIR A haplotype, and encodes either full-length (*KIR2DS4f*) or deleted (*KIR2DS4d*) allele variants. A deleted *KIR2DS4*

Abbreviations: AFND, Allele Frequencies Net Database; HSCT, hematopoietic stem cell transplantation; GVH, graft versus host; KIR, killer cell immunoglobulin-like receptors; GVHD, graft versus host disease; NK, natural killer cell; HLA, human leukocyte antigen; OS, overall survival.

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allele variant differs from the normal (full-length) *KIR2DS4* sequence in a 22 bp deletion in exon 5, which causes a frame shift, yielding a truncated *KIR2DS4* protein with a loss of the transmembrane and cytoplasmic domains of the full-length *KIR2DS4* protein. Such a truncated *KIR2DS4* protein is not anchored to the cell membrane and is potentially secreted in soluble form [6].

Interactions between KIR of donor cells and corresponding HLA class I molecules on the target recipient cells regulate the alloreactivity of NK cells. In allogeneic hematopoietic stem cell transplantation (HSCT), KIR-ligand mismatches in the graft-versus-host (GVH) direction trigger donor-versus-recipient NK-cell alloreactivity [7]. When the donor expresses an inhibitory KIR that fails to interact with its ligand in the recipient, the NK cells are activated through their corresponding activating KIRs or some other NK cell receptors with activating function. The presence of donor-derived alloreactive NK cells in the recipient is one potential factor affecting HSCT outcome since they play a major role as anti-leukaemia effector cells which kill recipient hematopoietic target cells as well as the leukaemia cells [8].

The first goal of this research was to investigate the polymorphism of KIR genes in patients with hematological malignancies in comparison with a healthy control group and determine their possible correlation with diseases. Since the given results pointed to the difference of the *KIR2DS4* gene as statistically significant, the next step of this research was to explore the possible impact of *KIR2DS4* gene on HSCT outcome. Since the NK cells regenerate the donors' KIR receptor repertoire in patients after HSCT, we assessed the impact of donors *KIR2DS4* allelic variants on overall survival rates (OS), non-relapse mortality (NRM), graft-versus-host disease (GVHD) incidence and relapse rate in patients who underwent related or unrelated HSCT. Based on these results we could determine whether the resolution of *KIR2DS4* allelic variants is helpful for donor selection in patients scheduled for HSCT. This is the first study of KIR genes association with HSCT outcome of transplanted patients in Croatia.

2. Subjects and methods

2.1. Study population

The study population consisted of 111 patients (adults: N = 91; children: N = 20) with hematological malignancies and their respective donors who underwent HSCT in the period of 2009–2011 at the University Hospital Centre Zagreb at the Department for Internal Medicine, Division of hematology. All the patients were undergoing transplantation for acute myelogenous leukaemia (AML, N = 41), acute lymphatic leukaemia (ALL, N = 28), chronic myelogenous leukaemia (CML, N = 6), chronic lymphatic leukaemia (CLL, N = 4), myelodysplastic syndrome (MDS, N = 7), non-Hodgkin lymphoma (NHL, N = 9), Hodgkin lymphoma (HL, N = 7) and other malignancies (N = 9).

Fifty-five patients (20 women and 35 men) were transplanted from a 10/10 HLA (HLA-A, -B, -C, -DRB1, -DQB1) allele-matched related donor, while for 56 patients (23 women and 33 men) the graft was obtained from a 10/10, 9/10 or 8/10 HLA allele-matched volunteer unrelated donor. All patient-donor pairs were HLA typed at the high resolution level using the standard PCR-SSP protocol for Olerup SSP® typing kits (Olerup GmbH, Vienna, Austria) at the Tissue Typing Centre, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb. The majority of the patients were treated with reduced-intensity conditioning (RIC) regimen mainly based on fludarabine in a dose of 30 mg/m^2 daily over 4–6 days, IV busulfan 3.2 mg/kg daily over 2–3 days, and anti-thymocyte globulin (ATG) (Thymoglobuline; Genzyme, Lyon, France) in a total dose of 5 mg/kg infused over 2 days. Other patients were treated with myeloablative conditioning regime receiving Busilvex in a daily dose of 4 × 0.8 mg/kg over 4 days, 60 mg/kg of cyclophosphamide over 2 days and ATG in total dose of 1.5 mg/kg over 11 h. GVHD prophylaxis was performed with cyclosporin A (CSA) alone after related HSCT or with CSA and mycophenolate mofetil (MMF) after unrelated HSCT. Patients received bone marrow grafts or peripheral blood stem cell grafts, mobilized from donors with granulocyte-colony-stimulating factor (GCSF) (10 μg/kg per day). No manipulation of the graft, such as T-cell depletion, was performed in any of the cases. The characteristics of the patients and their donors are given in Table 1.

2.2. KIR genotyping

All blood samples from the patients and their donors were collected during HLA typing at the Tissue Typing Centre Zagreb. Following the approval of the ethical committee of the Medical University of Zagreb and the ethical committee of the University Hospital Centre Zagreb, KIR genotyping was performed. Genomic DNA from whole blood containing EDTA was isolated with the NucleoSpin_Blood commercial kit (Macherey–Nagel, Duren, Germany). KIR genotyping was performed by the PCR-SSO method using at that time commercially available *Gen-Probe Lifecodes KIR-SSO typing kit* (at present: Immucor Transplant Diagnostics, Inc, Stamford, USA) containing 20 different oligonucleotide probes for known KIR genes or alleles (*KIR3DP1* and *KIR3DL3* as positive controls, *KIR2DL1*, *KIR2DL2*001-3/5*, *KIR2DL2*004*, *KIR2DL3*, *KIR2DL4*, *KIR2DL5*, *KIR2DP1*, *KIR3DL1*, *KIR3DL2*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4**whole exon 4, *KIR2DS4**whole exon 5, *KIR2DS4**-deleted exon 5, *KIR2DS5*, *KIR3DS1*, *KIR3DS1*049N*). The amplicons were quantified on the Luminex LABScan™ 100 flow analyzer (Luminex Corporation, Austin, TX, USA) and analyzed using the Quick-Type for Lifecodes software (version 3.3, Gen-Probe Transplant Diagnostics Inc. (at present: Immucor Transplant Diagnostics), Stamford, CT, USA) for generating KIR data. The typing determined the presence or absence of KIR genes and provided information about *KIR2DS4* allele variants. The distinguishing of *KIR2DS4* full-length and deleted allele variants is enabled with three probes in the test named and specified as follows: probe 145: *KIR2DS4*all full length,* probe 175:*2DS4*full length Ex.5* and probe 234: *2DS4*deletion Ex.5*.

2.3. Study design

The frequencies of KIR genes among 111 patients who underwent HSCT were determined and compared to the previously published frequencies of KIR genes in the control group (N = 121). The cohort of 111 patient-donor pairs was divided into two sub-groups according to HSCT setting: related HSCT (N = 55) and unrelated HSCT (N = 56). The primary end points of the study were overall survival (OS) rate, non-relapse mortality (NRM), GVHD occurrence and relapse rate and in each group we separately evaluated the effect of the *KIR2DS4* gene on those parameters. The individuals were considered positive for the *KIR2DS4* gene if the positive reaction was detected on probe 145 or both on probe 145 and probe 175, while they were considered as negative if they carried only a deleted *KIR2DS4* allele variant (positive reaction only for probe 234) or no *KIR2DS4* allele at all (all three probes with a negative reaction).

The starting point for time-to-event analysis was "date of transplantation". Overall survival (OS) rate was defined as the time to death from any cause. Surviving patients were censored at the time of last follow-up. Non-relapse mortality (NRM) was defined as all causes of death without evidence of initial disease. Acute and chronic GVHD were diagnosed according to standard criteria.