Prognostic and predictive role of hereditary genetic variants in multiple myeloma

Ph.D. thesis

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1. Introduction

Multiple myeloma (MM) is a malignant hematologic disease characterised by the transformation and the proliferation of post-germinal center-derived B cells. Based on primary genetic defects, MM can be categorized into two main subgroups: (i) MM with translocations involving the immunoglobulin heavy chain (IgH) locus and (ii) MM with hyperdiploidy. The diagnosis of MM is based on the criteria issued by the International Myeloma Working Group (IMWG). In the treatment protocols of myeloma beside immunomodulatory drugs (IMiD) and proteasome inhibitors (PI), which are still the backbone of treatment today, newer therapeutic tools have emerged such as monoclonal antibodies, checkpoint inhibitors and immunotherapies. Myeloma has an extremely heterogeneous disease course. A large number of prognostic and predictive factors have been described to explain the heterogeneity. Prognostic markers provide information about the outcome regardless of treatment applied. Important prognostic host factors are age and performance status. The International Staging System

(ISS) based on serum albumin and beta-2 microglobulin, is a prognostic marker to characterize the activity of myeloma and the induced organizational damage. The revised-ISS (R-ISS) shows improved prognostic power compared with the individual ISS, with the addition of the presence of cytogenetic abnormalities, and increased lactate dehydrogenase (LDH). In comparison with prognostic markers, predictive markers provide information specifically about different drugs or regimens and about the likelihood of response and outcome. Numerous biomarkers provide both prognostic and predictive value, including cytogenetics from routine diagnostics, as well as gene expression studies, which are still considered as research tools.

Whole genome association studies (GWAS) are powerful research tools for systematic screening and identification polymorphisms without the previous knowledge of gene and variants function that may play prognostic or predictive roles in a given disease.

In the first GWAS, a *FOPNL* (fibroblast growth factor receptor 1 oncogene partner N-terminal like) gene single nucleotide polymorphism rs72773978 was identified as a novel adverse prognostic factor for overall survival. The genetic variant presumably associate with increased expression of FOPNL, with increased amount of centrosomal proteins, which may affect the development of various centrosome abnormalities (centrosome amplification, abnormal centrosome structure and function), resulting shorter survival.

Regarding myeloma it was demonstrated that centrosome amplification was common in all stages of MM including monoclonal gammopathy of undetermined significance and is probably integral to disease pathogenesis. Thus it plays an early role in myeloma pathogenesis, and may associate with an adverse prognosis.

The backbone of myeloma treatment, bortezomib inhibits the proteolytic subunit (β5) of the proteasome, causing intracellular protein accumulation, consequential apoptosis of myeloma cells. In the proteasome subunit beta type 1 (*PSMB1*) gene, encoding the β6 subunit, rs12717 polymorphism was reported as a biomarker

influencing progression-free survival benefit in relapsed follicular lymphoma patients treated with bortezomib-rituximab or rituximab monotherapy. Genetic variants in proteasome genes may affect proteasome assembly, structure, function, and the conformation of drug binding sites of the proteasome.

2. Aims

The aim of our study was to examine *FOPNL* and *PSMB1* polymorphisms in a clinically well-characterized group of MM patients.

1) In case of *FOPNL* rs72773978 we aimed to investigate whether the presence of variant allele influence clinical characteristics (gender, age, ISS, cytogenetic abnormalities) in patients with MM.

2) To explore the predictive effect of *FOPNL* rs72773978 (calculation of overall- and progression-free survival in subgroups of differentially treated MM patients).

3) To genotyping of *PSMB1* rs12717 in MM patients in comparison with clinical characteristics of different genetic variants.

4) To study the predictive effect of *PSMB1* rs12717.

5) In case of the functional studies performed from samples of healthy individuals, we investigated the effect *PSMB1* rs12717 polymorphisms on proteasome structure, proteolytic activity with or without bortezomib inhibition.

3. Methods

3.1 Patients

In our study, we examined the *FOPNL* rs72773978 polymorphism in a group of 373 patients with MM diagnosed between 2005 and 2013. To determine the allele frequency of the rs72773978 polymorphism in Hungary, the control group consisted of 112 healthy blood donors. Regarding the *PSMB1* rs12717 polymorphism, 211 MM patients diagnosed between 2007 and 2013 were included in the study. Patients were diagnosed and followed in the Central Hospital of Southern Pest. Proteasome and *PSMB1* expression and function were studied in peripheral blood samples of 7 healthy volunteers.

3.2 Molecular genetic studies

Genotyping of *FOPNL* rs72773978 and *PSMB1* rs12717 were performed by real-time PCR followed by melting curve analysis on LC 480II (Roche) from genomic DNA.

3.3 Investigation of proteasome expression and function

The studies were performed at the Natural Sciences Research Center of the Eötvös Lóránd Research

Network. The quantity of 20S proteasome as well as PSMB1 expression were examined with a FACSCanto II flow cytometer. In the course of proteasome function assay, chymotrypsin-like, caspase-like, and trypsin-like activities were determined with the Proteasome Activity Fluorometric Assay Kit II (UBPBio) according to the manufacturer's instructions.

3.4 Fluorescent in situ hybridization assay (FISH)

The experiments were performed in the Cytogenetics Laboratory of the Central Hospital of Southern Pest. FISH results were available in 194 of 211 patients in the *PSMB1* and in 346 of 373 patients in the *FOPNL* study.

4. Results

4.1. FOPNL rs72773978 polymorphism

In the *FOPNL* rs72773978 study minor allele frequency in the myeloma group (AF ± 95%CI: 6,3 ± 1,8%) did not differ from the healthy control group (6,3 ± 3,2%). Due to the low number of homozygous TT individuals, further calculations were performed according to the dominant model: genotypes AT and TT were grouped together as minor variant carriers. In our studies, the following clinical characteristics were taken into account: gender, age, ISS, cytogenetic alterations, however, we did not find any differences concerning the variant allele carrier frequencies.

In our study group, the majority of patients (n = 241, 64.6%) received bortezomib-based therapy, while other patients (n = 132, 35.4%) received IMID-based treatment. We observed higher minor allele carrier frequency in the PI group than in the non-PI group (15.4% vs. 6.1%, p=008).

In the case of further subgrouping, the frequency of the minor allele did not differ in the different therapeutic

protocols. Considering the whole group in terms of responses to therapy, there was no difference between wild-type and *FOPNL* variant carriers, however, in the bortezomib-based therapeutic subgroup, significantly more *FOPNL* carriers achieved complete remission (CR) compared to wild-type patients (66.7% vs. 47.7%; p=0.047).

Examining the effect of the *FOPNL* rs72773978 polymorphism on progression-free (PFS) and overall survival (OS), we found that the variant allele had no effect on either PFS or OS (PFS: p=0.135; OS: p=0.328). Since the presence of the polymorphism showed a statistical interaction with bortezomib treatment for both PFS and OS (PFS: p=0.011; OS: p<0.001), the effect of polymorphism on outcome in the different treatment subgroups was also examined. Interestingly, the adverse effect of the variant allele described in the literature has changed depending on the treatment used. In the subgroup with non-PI-based therapy, carriership of the minor allele was significantly associated with adverse PFS and OS (PFS: p=0.042; OS: p=0.022). While in patients receiving PI-based treatment, the opposite trend

was observed. Regarding OS carriership of the variant allele was associated with better outcome and there was no difference in the case of PFS (OS: p=0.048; PFS: p=0.082). Considering sex, age, ISS and ASCT (autologous stem cell transplantation) as covariates, multivariate survival analyses indicated *FOPNL* rs72773978 carriership as an independent adverse risk factor for survival in the non-PI treatment group (PFS: p=0.33; HR: 1.58; 95%CI: 0.63-3.98; OS: p=0.045; HR: 2.69; 95%CI: 1.02-7.11). But we observed a trend in the opposite direction in the PI-treated subgroup (PFS: p=0.08; HR: 0.69; 95%CI: 0.46-1.04; OS: p=0.09; HR: 0.58; 95%CI: 0.31-1.09).

4.2 PSMB1 rs12717 polymorphism

The minor allele frequency of *PSMB1* rs12717 polymorphism (AF: 37.7 ± 4.7%) did not differ from that described in the literature (AF: 40.7 ± 7.0%). There were no differences in the clinical parameters of the patient groups (age, gender, ISS, cytogenetic abnormalities) between different genotype groups.

Considering therapy, variant allele frequency was significantly lower in the bortezomib-based group (n = 154, 72.9%) (55.8% vs. 71.9% p=0.05). In the case of different therapeutic protocols and in terms of responses to therapy there were no differences between for genotype groups. Next we focused on survival endpoints. Considering the whole patient cohort, patients with C/G and G/G genotypes experienced adverse relapse-free survival, while OS was not influenced (PFS: p=0.002; OS: p=0.268). Following this we examined PFS and OS for each subgroup (ISS, cytogenetics, treatment, ASCT). In the ISS stratified subgroups carriership of the minor allele was associated with adverse PFS, this effect was also observed in a subgroups of patients with a standard risk of cytogenetic abnormality, bortezomib treatment and ASCT. Analyzing OS only the ISS3 subgroup showed a difference. In multivariate analysis, besides age, ISS, FISH, bortezomib treatment, and ASCT, G/G genotype was also a significant risk factor for shorter PFS. Regarding OS, a significant difference between the C/C

and C/G genotypes was found, but not in the comparison of homozygous genotypes.

The quantitative analysis of proteasomes and the expression level of PSMB1 and functional assay for rs12717 polymorphism were determined from white blood cells obtained from peripheral blood of healthy individuals with C/C or G/G genotype. No difference was observed in proteasome quantities or PSMB1 expression were assessed specifically in various types of nucleated peripheral blood cell types; such as monocytes, T cells, B cells, and granulocytes. Chymotrypsin-, trypsin-, and caspase-like protease activities of the 20S subunit of the proteasome were lower in patients carrying the G/G variant allele. Regarding in vitro inhibitory effect of bortezomib, no difference in chymotrypsin-like activity of the proteasomes was observed between different genotypes (IC_{50}G/G: 0.146 vs. IC_{50}C/C: 0.150 μM). Decreased trypsin-like and caspase-like proteolytic activity was observed in the variant allele (G/G) when the activities were blocked by bortezomib (trypsin-like: IC_{50}G/G:

44.4 vs. IC_{50}C/C: 462 μM; caspase-like: IC_{50} G/G: 2.05 vs. IC_{50}C/C: 6.07 μM).

5. Conclusions

The allele frequency of the *FOPNL* rs72773978 polymorphism in myeloma patients did not differ from the results described in the literature, nor from healthy Hungarian individuals studied. In the examination of clinical characteristics, minor allele carrier frequency did not differ from the wild type in either case. When analyzing treatment protocols we observed higher minor allele carrier frequency in the bortezomib treatment group compared to the immunomodulatory group.

The *FOPNL* rs72773978 polymorphism showed a difference depending on the treatment used concerning overall survival and progression-free survival. Carriership of the minor allele was associated with adverse outcome in the non-bortezomib-based treatment group regarding PFS and OS. In the case of bortezomib-based treatment, this effect was reversed and the variant allele associated with favourable OS, while in the case of PFS there was a tendency. In multivariate analysis *FOPNL* rs72773978 carriership was an independent adverse risk factor for overall survival in the non-PI treatment group.

On the basis of our study the *FOPNL* rs72773978 polymorphism may have an effect not as a prognostic but as a predictive marker for multiple myeloma.

The allele frequency of the missense *PSMB1* rs12717 variant did not differ from the reported data. No differences were observed in the analysis of the different genotype groups and clinical characteristics.

In the whole group of patients *PSMB1* rs12717 polymorphism was associated with adverse PFS ($p=0.002$), which also proved to be an independent risk factor by multivariate analysis (PFS: $p<0,001$; HR: 2.293; 95%CI: 1.458-3.604). The OS was not affected by the presence of the minor allele.

In the functional study of the *PSMB1* rs12717 polymorphism the number of proteasomes and the expression of PSMB1 did not differ in the samples of healthy individuals with C/C and G/G genotypes). Lower chymotrypsin, trypsin, and caspase-like 20S proteasome proteolytic activities were observed in individuals carrying the minor allele in homozygous form. When

proteolytic activities were inhibited by bortezomib decreased IC_{50} values were demonstrated for trypsin and caspase-like activities of healthy subjects with G/G genotypes but not for the chymotrypsin-like activity.

These results suggest that *PSMB1* rs12717 can be both a prognostic and on the basis of functional study a predictive marker in MM.

6. List of publications

6.1 Publications related to the present thesis

- **Kiss KP**, Varga G, Mikala G, Balassa K, Bors A, Kovy P, Meggyesi N, Kozma A, Csacsovszki O, Remenyi P, Valyi-Nagy I, Tordai A, Masszi T, Andrikovics H. The adverse effect of FOPNL genomic variant is reversed by bortezomib-based treatment protocols in multiple myeloma.Leuk Lymphoma. 2018 Mar;59(3):710-716.
- Varga G, Mikala G, **Kiss KP**, Kosóczki É, Szabó E, Meggyesi N, Balassa K, Kövy P, Tegze B, Szombath G, Tordai A, Andrikovics H, Homolya L, Masszi T. Proteasome Subunit Beta Type 1 P11A Polymorphism Is a New Prognostic Marker in Multiple Myeloma. Clin Lymphoma Myeloma Leuk. 2017 Nov;17(11):734-742.

6.2 Publications not related to the present thesis

- Balassa K, Andrikovics H, Remenyi P, Batai A, Szilvasi A, Bors A, **Kiss KP**, Rajczy K, Inotai D, Torbagyi E, Lengyel L, Barta A, Gopcsa L, Tordai A, Masszi T. Sex-specific survival difference in association with HLA-DRB1∗04 following allogeneic

haematopoietic stem cell transplantation for lymphoid malignancies.Hum Immunol. 2018 Jan;79(1):13-19.

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associated thrombotic microangiopathy. Bone Marrow Transplant. 2015 Oct;50(10):1321-5.

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- Tordai A, Bors A, **Kiss KP**, Balassa K, Andrikovics H, Batai A, Szilvasi A, Rajczy K, Inotai D, Torbagyi E, Lengyel L, Barta A, Remenyi P, Masszi T. Donor KIR2DS1 reduces the risk of transplant related mortality in HLA-C2 positive young recipients with hematological malignancies treated by myeloablative conditioning. PLoS One. 2019 Jun 25;14(6):e0218945.