Leukemia Research 39 (2015) 1462–1466

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Polymorphisms within beta-catenin encoding gene affect multiple myeloma development and treatment

Aleksandra Butrym[a,b], Justyna Rybka[a], Piotr Łacina[c], Katarzyna Gębura[c], Diana Frontkiewicz[d], Katarzyna Bogunia-Kubik[c,d,*], Grzegorz Mazur[d]

[a] Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, Poland
[b] Department of Physiology, Wroclaw Medical University, Wroclaw, Poland
[c] Laboratory of Clinical Immunogenetics and Pharmacogenetics, L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
[d] Department of Internal, Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Wroclaw, Poland

ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form
25 September 2015
Accepted 14 October 2015
Available online 19 October 2015

Keywords:
CRBN gene polymorphism
CTNNB1 gene polymorphism
Multiple myeloma
Stage of the disease
Response to immunomodulating therapy

ABSTRACT

Recent studies have suggested that cereblon (*CRBN*) is essential for the anti-myeloma (MM) activity of immunomodulatory drugs (IMiDs), such as thalidomide and lenalidomide, and that dysregulation of Wnt/β-catenin pathway may be one of possible reasons of lenalidomide resistance. This prompted us to analyze the effect of polymorphisms within the genes coding for cereblon (*CRBN* (rs121918368 C > T)) and β-catenin (*CTNNB1* (rs4135385 A > G; rs4533622 A > C)). MM patients ($n = 142$) and healthy individuals ($n = 123$) were genotyped using the Light SNiP assays.

The presence of the *CTNNB1* (rs4533622) *A* allele was more frequently detected in patients presented with stage II–III disease according to International Staging System (63/82 vs. 26/44, $p = 0.043$) and Durie–Salmon criteria (75/99 vs. 14/26, $p = 0.049$).

The *CTNNB1* (rs4135385) *AA* homozygosity was more frequent among patients with better response to CTD, i.e. cyclophosphamide–thalidomide–dexamethasone (18/23 vs. 32/60, $p = 0.047$). Patients carrying the *CTNNB1* (rs4533622) *AA* genotype were better responders to the first line therapy with thalidomide containing regimens ($p < 0.05$). No significant association was observed between the effect of lenalidomide therapy and polymorphisms studied. However, the occurrence of neutropenia during lenalidomide therapy was more frequent among the *CTNNB1* (rs4135385) *AA* carriers ($p = 0.019$), while the *CTNNB1* (rs4533622) *AA* homozygosity characterized patients with high grade (3–4) neutropenia ($p = 0.044$).

No association was found for the *CRBN* polymorphism.

These results suggest that the *CTNNB1* polymorphisms may affect the clinical course and response to chemotherapy in patients with multiple myeloma.

1. Introduction

Multiple myeloma (MM) is a plasma cell malignancy derived from an early precursor of the B-cell lineage characterized by bone marrow infiltration, lytic bone lesions and the presence of a monoclonal protein in serum and/or urine. MM accounts for 1% of all malignancies in Caucasians and is rare in patients younger than 30 and its incidence increases in the elderly. This disease belongs to the group of lymphoproliferative disorders with heterogeneous clinical course.

Thalidomide and its derivatives, lenalidomide and pomalidomide, known as immunomodulatory drugs (IMiDs), are novel drugs used in MM treatment that significantly increases survival rates in patients [1]. Mechanisms underlying immunomodulatory and teratogenic effects of thalidomide have been unknown until a novel protein, cereblon, has been recognized as its molecular target.

When cereblon was first discovered it had been named after its presumed role in cerebral development as well as presence of Lon domain [2]. Thalidomide binds strongly to cereblon, thereby interfering with the E3 ubiquitin ligase functions [1,3]. Thalidomide exerts pleiotropic effects on myeloma cells including an up-regulation of $p21^{WAF-1}$ and down-regulation of interleukin-8, both of which cause G0/G1 arrest. The teratogenicity of thalidomide is thought to be a result of fibroblast growth factor-8 (FGF-8) down-regulation [4,5].

* Corresponding author at: L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wroclaw, Poland. Fax: +48 71 3371382.
E-mail address: bogunia@iitd.pan.wroc.pl (K. Bogunia-Kubik).

http://dx.doi.org/10.1016/j.leukres.2015.10.007
0145-2126/

A major problem in thalidomide/lenalidomide treatment is that MM cells tend to develop drug resistance. This process has not, as of yet, been fully elucidated, but dysregulation of Wnt/β-catenin pathway has been observed in lenalidomide-resistant cells. Even though β-catenin has been named for its structural function in β-catenin–cadherin adherens junctions, it is also a key element in a major nuclear signaling pathway called Wnt pathway [6]. In this pathway transcription co-activator β-catenin is phosphorylated by an Axin/CK1α/APC/GSK3 complex and eventually degraded, but the complex is disassembled once the regulatory protein Wnt binds to the Frizzled receptor; subsequent accumulation of β-catenin and its translocation to nucleus causes increased cell survival [7,8]. It is thus presumed that disruption of E3 ubiquitin ligase complex functionality after intensive thalidomide treatment causes β-catenin accumulation even when Wnt is bound to the Frizzled receptor, thus causing overexpression of various pro-survival and anti-apoptotic factors that may be responsible for the thalidomide/lenalidomide resistance [9].

In accordance with the role of beta-catenin in cell proliferation and survival, several mutations in the Wnt pathway have been linked to cancer development, although none of them has, as of yet, been associated with multiple myeloma [10].

As the recent studies have suggested that *CRBN* is essential for the anti-myeloma activity of immunomodulatory drugs, such as thalidomide and lenalidomide and that dysregulation of Wnt/β-catenin pathway may be one of the possible reasons of lenalidomide resistance, our present study aimed to analyze the effect of the three single nucleotide polymorphisms (SNPs) within the genes coding for cereblon (*CRBN* (rs121918368 C > T)) and β-catenin (*CTNNB1* (rs4135385 A > G; rs4533622 A > C)) on disease susceptibility and response to chemotherapy in MM patients.

Cereblon encoding gene (*CRBN*) in humans is located on chromosome 3p26.2 and is composed of 11 exons. The *CRBN* rs121918368 single nucleotide polymorphism (SNP) was reported in 2004 [2]. This SNP refers to cytosine to thymine substitution in exon 11, which is equivalent of a change in the arginine codon to stop codon and formation of a shorter protein [2,11].

Human β-catenin gene (*CTNNB1*) is located on chromosome 3 and straddles the border between 3p22 and 3p21.3 regions [12]. It consists of 16 exons separated by short introns, whose length does not exceed 550 base pairs [13]. The rs4135385 *CTNNB1* polymorphism is a substitution of adenine for guanine in intron 13 close to exon 14. Thus, due to its position, this SNP may potentially affect the splicing of β-catenin mRNA [14]. The other *CTNNB1* polymorphism, rs4533622, is an adenine to cytosine substitution which is also located within the intronic sequence.

2. Materials and methods

2.1. Patients and controls

One hundred and fifty patients (pts) aged 37–85 years (median age 61), diagnosed with multiple myeloma, were included into the study. There were 68 men and 82 women. In 15 cases diagnosis of multiple myeloma was proceeded by MGUS (monoclonal gammopathy of undetermined significance). There were 20 patients with renal insufficiency. Clinical characteristic of patients is presented in Table 1.

In addition 123 healthy individuals of both sexes (F/M = 60/63) served as a control group for the polymorphism study that involved 142 patients.

2.2. Immunomodulating therapy and response

2.2.1. Thalidomide

One hundred and fifteen patients were treated with thalidomide containing regimens (90% with CTD: cyclophosphamide, thalidomide, dexamethasone). Responses to first line chemotherapy in patients were as follows: complete response (CR) 18 pts, very good partial response (VGPR) 20 pts, partial response (PR) 70 pts, stable disease (SD) 13 pts, progressive disease 19 pts, in 10 pts evaluation of response was not assessed. In 76 patients, after first line therapy, megachemotherapy supported by autologous stem cell transplantation (autoBMT) was performed. Responses after autoBMT were as follows: 30CR, 28 VGPR, 18 PR. 47 progressed after autoBMT.

Table 1
Patients' characteristics.

Data	Number of patients
Age	
≥50 years	129
<50 years	21
Sex	
Male	68
Female	82
Stage according to Durie–Salmon	
I	26
II	54
III	70
International staging system (ISS)	
I	44
II	44
III	62
Myeloma type	
IgG	83
IgA	31
Light chain disease (LCD)	27
Non-secretory	6
Solitary	3
Beta-2-microglobulin (B2M)	
≤norm	16
>norm	134
Anemia	
<10 g/dl	38
≥10 g/dl	102

Median progression free survival after induction chemotherapy was 14 months (range 0–72 months). We observed 25 cases of thalidomide induced neuropathy and 10 cases were complicated with thrombotic events while on thalidomide.

2.2.2. Lenalidomide

From whole analyzed population 33 patients were treated with lenalidomide–dexamethasone (Len–Dex) regimen, in majority of them as a third line therapy. Median number of cycles administered was 6 (range 2–38 cycles). In 17 pts grade 3 neutropenia was reported. In case of two patients therapy was complicated by thrombosis. Responses to lenalidomide were as follows; CR in 5 cases, 9 cases of VGPR, 6 PR, 7 SD and 6 patients progressed while on lenalidomide. Median progression free survival was 5 months (range 3–31 months).

2.3. CRBN and CTNNB1 genotyping

DNA was extracted from samples of peripheral blood taken on EDTA using Maxwell 16 Blood DNA Purification Kit (Promega Corp., USA) or silica membranes (Qiagen, Germany) following the recommendations of the manufacturers. Determination of the (*CRBN* (rs121918368 C > T)) and β-catenin (*CTNNB1* (rs4135385 A > G; rs4533622 A > C)) polymorphisms was carried out by the LightSNiP typing assay (TIB-MolBiol, Berlin, Germany) on a LightCycler 480 Real-Time PCR system (Roche Applied Science, Mannheim, Germany). The amplifications were performed following the recommendation of the manufacturer.

2.4. Statistical analysis

Genotype and allele frequencies were compared between the study groups by the Chi^2 test with Yates correction or Fisher's exact test when necessary using Statistica 5.5 for Windows software. The odds ratio (OR) was calculated by Haldane's modification of Woolf's method and the significance of its deviation from unity was estimated by Fisher's exact test. Probability values <0.05 were considered statistically significant, and those between 0.05 and 0.1 as indicative of a trend.

3. Results

3.1. Distribution of the CRBN and CTNNB1 alleles and genotypes among patients with MM and healthy controls

The *CTNNB1* alleles and genotypes segregated similarly in patients with MM and healthy individuals. Thus none of the *CTNNB1* polymorphisms studied appeared to be associated with susceptibility to the disease. The details are presented in Table 2.