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657DEL5 MUTATION OF THE NBS1 GENE IN MYELODYSPLASTIC SYNDROME

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Abstract – Myelodysplastic syndromes (MDS) are clonal hematologic stem cell disorders with an as yet unknown molecular pathology. Genetic instability has been proposed as a cause of MDS. Mutations in the *NBS1* gene, whose product nibrin (p95) is involved in DNA damage repair and cell-cycle control, might be associated with an elevated predisposition to the development of MDS. The aim of the study was to examine truncating 5 bp deletion (657del5), the most frequent *NBS1* gene mutation in Slavic populations, in MDS patients. Among 71 MDS patients, we found one case that was heterozygous for the *NBS1* 657del5 mutation. To the best of our knowledge, this is the first report of a *NBS1* mutation in MDS.

Key words: MDS; nibrin; *NBS1* mutations; 657del5.

INTRODUCTION

The myelodysplastic syndrome (MDS) is characterized by ineffective hematopoiesis and elevated apoptosis in the bone marrow, resulting in peripheral blood cytopenias and a risk of progression to acute myeloid leukemia (AML).

The NBS1 gene encodes nibrin (p95), a member of the MRE11/RAD50 double-strand break repair complex (Carney et al., 1998). Protein p95 is important for the cellular response to DNA damage (Wu et al., 2000), activation of cell cycle checkpoints (Zhao et al., 2000), processing of recombination intermediates (Tauchi et al., 2002) and telomere maintenance (Lombard et al., 2000).

Mutations in *NBS1* are recognized as the main molecular event in the development of autosomal recessive chromosomal instability disorder, called Nijmegen breakage syndrome (NBS). The clinical features of NBS patients are microcephaly, growth retardation, a "bird-like" face, radiation sensitivity, immunodeficiency, and increased risk of developing cancer, especially lymphoid malignancies. Independently from Nijmegen breakage syndrome, alterations in *NBS1* DNA sequence are found in a number of malignancies. Deletion of 5 bp in exon 6 (657del5), resulting in a truncated protein, is the most frequent mutation in *NBS1* in Slavic populations.

The aim of the present study was to examine 657del5 mutation of *NBS1* in a cohort of 71 MDS patients and to determine the potential role of this genetic alteration in the pathogenesis of MDS.

MATERIALS AND METHODS

This study was conducted on 71 MDS patients diagnosed and treated at Institute of Hematology, Clini-

cal Centre of Serbia. Among the patients, 19 were classified as RA, 17 as RAEB-1, 12 as RAEB-2, 15 as RARS and 8 remained unclassified.

DNA was obtained from archived bone marrow microscope slide smears using a standard phenol-chloroform method.

The 657del5 mutation is a deletion of five nucleotides (ACAAA) that reside in exon 6 of the *NBS1* gene. To screen for 657del5, we used the polymerase chain reaction (PCR) assay described by Seeman (Seeman et al., 2004). A fragment from *NBS1* exon 6 was amplified using the following forward and reverse primers: 5'-AATGTTGATC TGTCAGGACG-3' and 5'-TATAAATGTTTTCCCTTTGAAGA-3' with annealing temperature at 56.5^{0}C. PCR products were analyzed on 8% polyacrylamide gels.

Individuals without the deletion had a 60 bp PCR fragment, whereas individuals heterozygous for deletion 657del5 displayed an additional 55 bp-long PCR product.

RESULTS AND DISCUSSION

Myelodysplastic syndromes are heterogeneous and their primary molecular pathology is still unknown. A predisposition to MDS can be caused by heterozygous mutations of different genes, such as *GATA2* (Hahn et al., 2011) or the genes *TERC* and *TERT* (Yamaguchi et al., 2003), whose products are involved in telomerase activity. Since genetic instability has been discussed as a cause of MDS, mutations in the *NBS1* gene, involved in DNA damage repair mechanisms and cell-cycle control, might also be associated with an elevated predisposition to the development of MDS.

The *NBS1* gene is mutated in the majority of patients with Nijmegen breakage syndrome (NBS). NBS belongs to a group of autosomal recessive chromosomal instability disorders, characterized by immunodeficiency, clonal occurrence of chromosomal rearrangements and hypersensitivity to irradiation. NBS patients have a risk of developing different malignancies, such as malignant lymphoma, acute lymphoblastic leukemia, glioma, medulloblastoma (van der Burgt et al., 1996) (Distel et al., 2003) and rhabdomyosarcoma (der Kaloustian et al., 1996) (Meyer et al., 2004). *NBS1*-heterozygous individuals also have an elevated risk of developing malignancies, in particular non-Hodgkin's lymphoma, lymphoblastic leukemia, breast, prostate and colorectal cancers (di Masi, 2008).

The *NBS1* gene product nibrin (p95) is a member of the MRE11/RAD50/p95 protein complex involved in DNA double-strand break repair. This complex is among the earliest respondents to DNA double-strand breaks with critical roles in recognition, stabilization of damage and initiation of cell-cycle checkpoint signaling cascades (Williams et al., 2007)

Due to impaired DNA repair, *NBS1*-heterozygous individuals have higher spontaneous and induced chromosome instability. Carriers of *NBS1* mutation display a 3-fold higher rate of chromosome translocations compared with non-carriers (Stumm et al., 2001). In addition, heterozygous mutation of the *NBS1* gene can significantly affect natural variation in gene expression (Cheung, 2006).

Experimental support that *NBS1* heterozygosity might contribute to bone marrow failure comes from studies with animal models. The induction of an *NBS1*-null mutation in mice led to increased chromosome damage, radiomimetic sensitivity and dramatic decrease in cell survival in the bone marrow, thymus and spleen (Demuth et al., 2004). Additionally, mice in which the C-terminal domain of nibrin was deleted showed severe apoptotic defects in multiple tissues, including hematopoietic cells (Stracker et al., 2007).

RAD50, MRE11 and NBS1 are associated with the telomeric repeat-binding factor, TRF2, during the S phase of cell cycle (Zhu et al., 2000), suggesting that p95 may have a role in telomere maintenance. Telomerases and the length of telomeres have an essential role in the maintenance of genomic integrity and the long-term viability of high-renewal organ

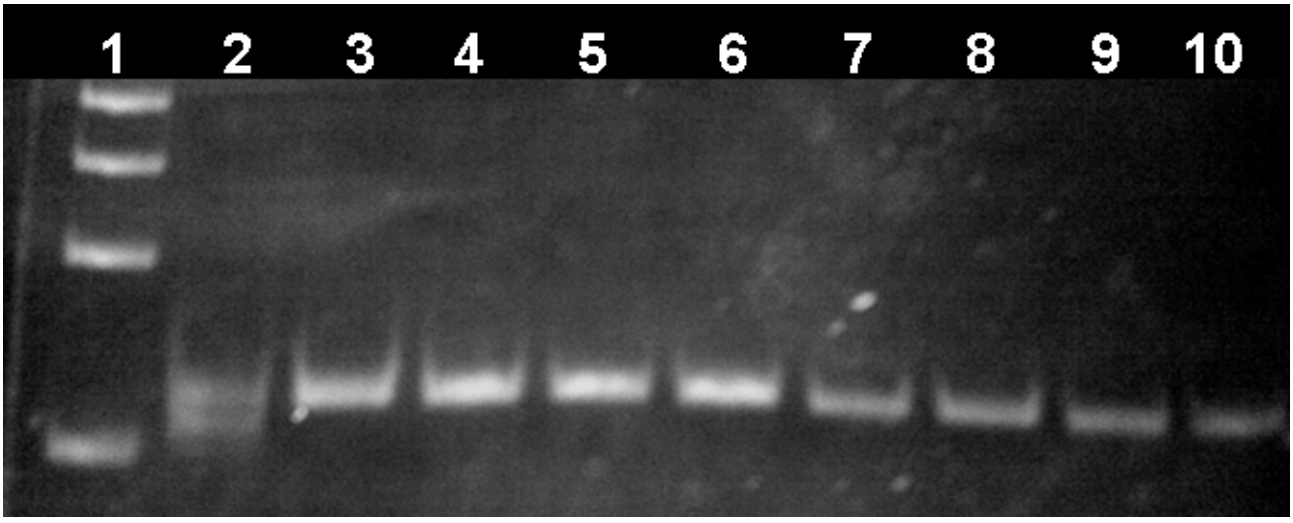

Fig. 1: 1 – DNA ladder; 2 – MDS patient with 657del mutation; 3 – 10 MDS patients

systems. In the telomerase-defective organism, the proliferative capacity of hematopoietic cells in the bone marrow is compromised (Lee et al., 1998). The radiosensitivity of telomere-dysfunctional cells correlates with delayed DNA break repair (Wong et al., 2000). Mutations of *TERC* and *TERT* genes, whose products are the main constituents of the telomerase complex, are considered to cause telomere shortening, impair the proliferative capacity of hematopoietic stem cells (Vulliamy et al., 2006) and contribute to the development of bone marrow failure (Yamaguchi et al., 2005). The *NBS1* gene involvement in telomere maintenance leads us to presume that *NBS1* deficiency and telomere dysfunction may act together, especially because the same correlation with the *ATM* gene has been established (Wong et al., 2003).

NBS1 has not yet been shown to be one of the genes that predispose to MDS; however, all these findings encouraged us to initiate a study to find out whether the *NBS1* gene is involved in the pathogenesis of this disease. We screened MDS patients for truncating 5-bp deletion (657del5) in exon 6 of the *NBS1* gene because it is the most common (Varon et al., 1998), and carriers of this mutation are very frequent in populations of Slavic origin (Varon et al., 2000). This frameshift mutation introduces a premature termination signal at codon 218 and results in a truncated polypeptide (Matsuura et al., 1998). Of all analyzed patients only one, classified as RARS MDS, was a carrier of the 657del mutation of the *NBS1* gene (Fig. 1). This individual was heterozygous for the major *NBS1* mutation, 657del5 (1.41%). As far as we know, this is the first report of *NBS1* mutation in MDS. Although the number of analyzed samples was small, our study indicates that the *NBS1* gene might represent a potential factor involved in the pathogenesis of MDS. A number of questions remain open, especially because mutations different from 657del5 have been identified in the *NBS1* gene. Further studies are necessary to clarify the biological significance of the *NBS1* gene in MDS.

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