RESEARCH ARTICLE

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The Frequency of *SF3B1* Mutations in Thai Patients with Myelodysplastic Syndrome

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Abstract

Genetic mutations in genes encoding critical component of RNA splicing machinery including SF3B1 are frequently identified and recognized as the pathogenesis in the development of myelodysplatic syndrome (MDS). In this study, PCR sequencings specific for SF3B1 exon 13, 14, 15, and 16 were performed to analyse genomic DNA isolated from bone marrow samples of 72 newly diagnosed MDS patients. We found that 10 of 72 (14%) patients harbor SF3B1 missense mutations including E622D (1/72), R625C/G (2/72), H662Q (1/72), K666T (1/72), K700E (4/72) and G740E (1/72), respectively. Mutations were predominantly located on exon 14 and 15 of SF3B1 coding sequence. Interestingly, patients with SF3B1 mutations exhibited higher platelet counts (195×10^9/L VS. 140×10^9/L, p-value = 0.025) as well as lower hemoglobin levels (81 g/L VS. 92 g/L, p-value = 0.009) and associated with ring sideroblast phenotype (p-value < 0.001) when compared with patients without the SF3B1 mutation. In summary, we reported the frequency of SF3B1 mutations in Thai patients with different subtypes of MDS. SF3B1 mutations were predominantly occurred in MDS-RS and considered as favourable prognosis value. This study further highlighted the clinical important of SF3B1 mutations analysis for the classification of MDS.

Keywords: Myelodysplastic syndrome- RNA splicing machinery- SF3B1 mutation

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Introduction

Myelodysplastic syndrome (MDS) is a group of chronic hematological malignancies characterized by ineffective hematopoiesis which is manifested by the morphological dysplasia in hematopoietic cells and by peripheral cytopenia (Arber et al., 2016). Additionally, functional abnormalities of blood cells were observed in patients such as predisposed to severe infections and serious bleeding (Issa, 2013). MDS are physically pre-leukemic condition which is frequently transformed into the terminally acute myeloid leukemia (AML) (Walter et al., 2012). MDS is relatively heterogeneous in clinical representation, prognosis, and treatment outcomes. Recently, the WHO classification of myeloid neoplasms and acute leukemia has classified subtypes of MDS based on the assessment of cytopenia, the morphological analysis, and the incorporation of genetic mutation data (Arber et al., 2016; Vardiman et al., 2009). During the last two decades, several studies revealed the heterogeneous spectrum of genetic alterations which are recognised as the key drivers for the initiation of MDS. Those are including mutations on genes involving in cellular signalling (e.g., JAK2, KRAS, and CBL), DNA methylation (e.g., IDH1/2, TET2, and DNMT3A), chromatin modification (e.g., ASXL1 and EZH2, transcriptional control (GATA, RUNX1, and EVI1), DNA damage respond (e.g., TP53), and recently RNA splicing process (e.g., *SF3B1*, U2AF1, SRSF2, and ZRSR2) (Beer et al., 2010; Bejar et al., 2011; Bejar et al., 2014; Boultwood et al., 2010; Cazzola et al., 2013; Ernst et al., 2010; Gelsi-Boyer et al., 2009; Green and Beer, 2010; Haferlach et al., 2014; Jadersten et al., 2011; Je et al., 2013; Makishima et al., 2010; Makishima et al., 2012; Malcovati et al., 2011; Nikoloski et al., 2010; Papaemmanuil et al., 2013; Patnaik et al., 2012a; Patnaik et al., 2013). The complexity of genetic mutations in individual MDS results in disease heterogeneity as represent with various degrees of disease severity,

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treatment outcome, and progression to acute myeloid leukemia.

Recent advance in deep-sequencing technology to analyse MDS samples was able to identify recurrent somatic mutations in gene encoding RNA splicing machinery (Papaemmanuil et al., 2011; Yoshida et al., 2011). These are including four common spliceosome mutations, *SF3B1*, U2AF1, SRSF2, and ZRSR2 which could be identified in nearly 70% of MDS patients (Bejar, 2016). Interestingly, those mutations were heterozygous missense mutations and represented as exclusive manner underlining the molecular mechanism of spliceosome in the initiation of MDS (Inoue et al., 2016). Additionally, spliceosome mutations have been reported in different types of leukemia and cancer such as in chronic lymphocytic leukemia (Wang et al., 2011), chronic myelomonocytic leukemia (Patnaik et al., 2013), de novo adult acute myeloid leukemia (Je et al., 2013), and uveal melanoma (Field and Harbour, 2014) with relatively rare frequency. While U2AF1 and SRSF2 are frequently mutated in advance subtypes of MDS and chronic myelomonocytic leukemia, *SF3B1* mutation is commonly detected in low-risk MDS with ring sideroblasts and associated with favourable outcome (Makishima et al., 2012). *SF3B1* coding gene is located on chromosome 2q33.1 and recognised as a member of U2 small ribonucleoprotein complex which is critical for pre-mRNA splicing during transcription (Patnaik et al., 2012b). Recent studies demonstrated that about 60 to 80 % of MDS-RS habor somatic mutations of *SF3B1* (Yoshida et al., 2011) (Malcovati et al., 2015; Patnaik et al., 2012b; Zhu et al., 2016). Interestingly, the mutation patterns of *SF3B1* coding sequence are not random and frequently observed at the exon 12 to 16. Hot spot mutations of *SF3B1* commonly identified in MDS are including K700E (~ 50%), K666N/Q/R, E622D, H662D/Q, Y623C and R625C/L. Collectively, the data highlights the potential use of *SF3B1* mutations as a biomarker for the molecular classification of MDS/MPN. In this work, we analysed the hotspot mutations of *SF3B1* in bone marrow samples of 72 patients with MDS. Moreover, we further described the clinical and laboratory data of newly diagnosed Thai patients with MDS that represented as a regional MDS database.

Materials and Methods

Patients and samples

A total of 72 bone marrow aspiration samples from newly diagnosed MDS patients at Ramathibodi Hospital, Bangkok, Thailand since January 2015 to September 2016 were included in this study. The diagnostic criteria was based on the 2008 or the recently revised 2016 WHO classification of myeloid neoplasms and acute leukemia. Other cytopenias without evidence of clonal hematopoiesis and other malignancies were excluded from this study. Demographic data and hematological findings of individual patients were recorded. Genomic DNA was purified by using QIAamp DNA Blood mini kit (Qiagen, Germany) according to the manufacturer's instruction and subsequently measured the concentration by using the Nanodrop 2000 spectrophotometer (Thermo Scientific, USA) according to the instruction protocol. This work was approved by the ethic committee on human right related to research involving human subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand and followed the principles of the Declaration of Helsinki (ID 06-59-63).

SF3B1 exon 13, 14, 15, and 16 mutations analysis

We performed the specific PCR followed by direct sequencing to detect hotspot mutations of *SF3B1* (exon13-16). The primers specific for the amplification and sequencing of *SF3B1* exon 13, 14, and 15 to 16 and expected PCR amplicon sizes were listed in table 1. The amplification was carried out in the Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The optimal PCR condition which could be applied to all analysed exons was following; initial denaturing at 95 ^{o}C for 10 minutes, 35 cycles of 94 ^{o}C for 45 seconds, 53 ^{o}C for 45 seconds, 72 ^{o}C for 45 seconds, and final elongation at 72 ^{o}C for 10 minutes. All PCR products were confirmed by 2% agarose gel electrophoresis. Sequencing reactions were performed using BigDye terminator V1.1 Cycle Sequencing Kit (Life Technologies) according to the manufacture instruction. The Sequencing products were run on the 3130 x l Genetic Analyzer (Applied Biosystem, Foster City, CA, USA) and subsequently analysed and compared with NCBI reference sequence: NG_032903.2 by using SeqScape® software version 2.5 (Thermo Fisher Scientific, USA).

Cytogenetic study

Complete cytogenetic study was performed at Human Genetic Laboratory, Department of Pathology, Ramathibodi Hospital using G-banding technique after short term culture without mitogen activation. On-screen karyotyping was performed on 20-30 metaphases using Ikaros software, MetaSystems, Germany. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2016).

Statistical analysis

The χ^2 test or Fisher's exact test was performed to determine statistical significance of associations between *SF3B1* mutations and categorical variables including sex, WHO classification, karyotypes, and IPSS-R risk classification. The comparisons of numerical variable including age, hematologic parameters between groups were performed by using Mann-Whitney U test for nonparametric data and Student's t-test for parametric data. All statistical calculations were conducted on the statistical package SPSS version 16.0 (SPSS, Inc, Chicago, IL, USA) software. All tests were two-sided comparison, and p-values less than 0.05 was considered to indicate statistical significance.

Results

Clinical and laboratory features of 72 MDS patients

A total of 72 newly diagnosed MDS patients were

Table 1. Primers for PCR Amplification and Direct Sequencing of *SF3B1* Exon 13, 14, and 15-16

Positions	Forwards (5' – 3')	Reverses (5' – 3')	Product size (bp)
SF3B1 Exon 13	GTACATGAGCATTTCATCAGTA	CAACCATTTCTTTCCATAATCA	222
SF3B1 Exon 14	ATTACCAACTCATGACTGTCC	TACATTACAACTTACCATGTTC	326
SF3B1 Exon 15-16	ATCTGGATGATATTGTGTAACT	CAGTTTACATTAACAAATCTGG	486

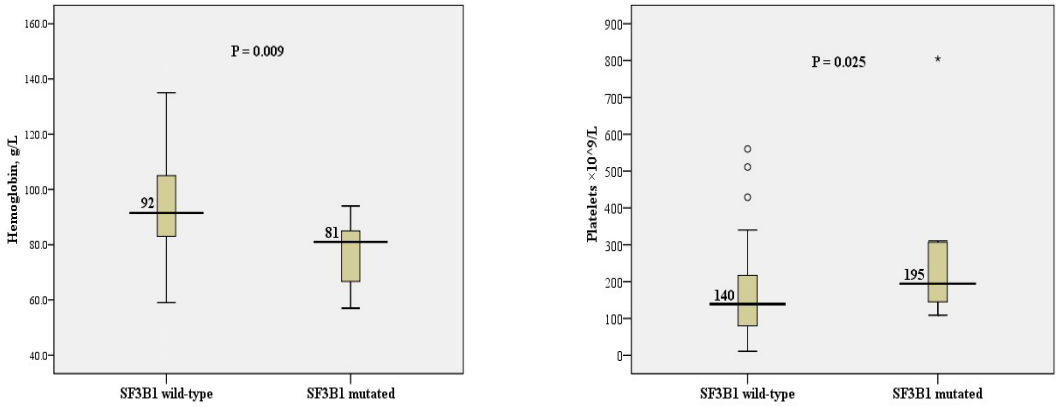

Figure 1. The Comparison of Hemoglobin Level and Platelet Number in MDS Patients with Wild-Type and *SF3B1* Mutation

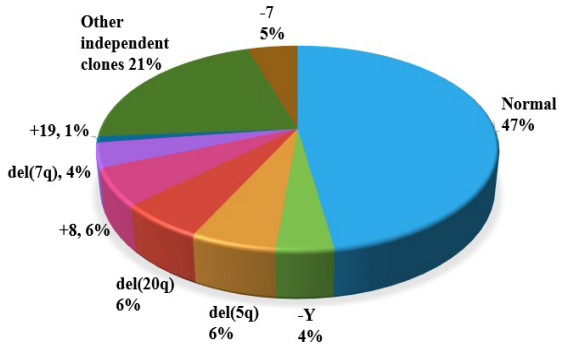

Figure 2. The Karyotyping Data of 72 MDS Patients Participating in This Study. The present of individual chromosomal abnormality in each patient was counted as one event.

included into this study. Those were including 21 MDS-SLD, 22 MDS-MLD, 6 MDS-RS, 1 MDS with isolated del(5q), 11 MDS-EB-1, 3 MDS-EB-2, 6 MDS-U, and 2 patients with secondary AML. The median age of patients at diagnosis was 72.5 years (range 24-94 years). There was no statistically significant different between the number of affected male and female (male/female ratio = 0.85). The clinical and laboratory characteristics of these patients regarding to their *SF3B1* statuses are presented in Table 2. Briefly, *SF3B1* exon 14 and 15 mutations were detected in 10 out of 72 MDS patients (14%). Interestingly, higher in platelet level ($p = 0.025$) as well as lower in hemoglobin level ($p = 0.009$) were observed in MDS who carried *SF3B1* mutations (Figure 1). In addition, bone marrow (BM) study revealed that 15 of 72 patients are evidently positive for ring sideroblast which ranged from 10 to 50%. Moreover, we found that *SF3B1* mutations were markedly associated with the present of BM ring sideroblast (8 out of 10 cases) ($p < 0.001$). Normal karyotype was identified in 34 patients

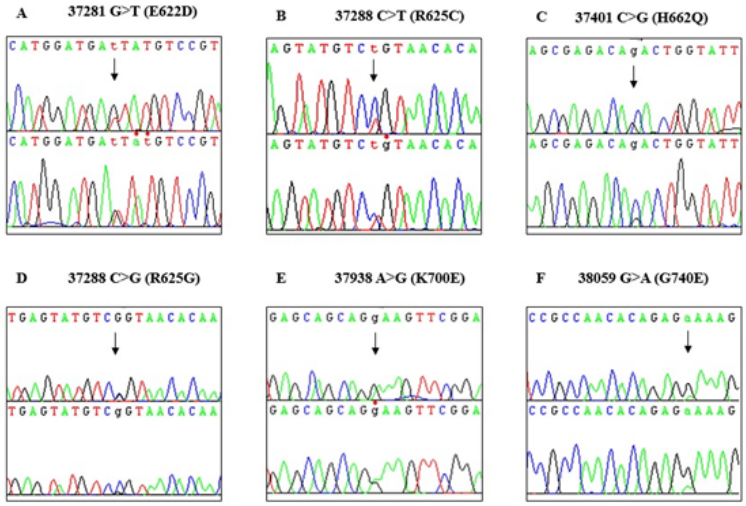

Figure 3. Sequencing Chromatogram of *SF3B1* Mutations with Indicated Genotypes

Table 2. Clinical and Laboratory Characteristics of 72 MDS Patients with SF3B1 Mutation Status

Variable	All patients with MDS (n=72)	MDS patients without the *SF3B1* mutation (n=62)	MDS patients with the *SF3B1* mutation (n=10)	P-value
Median age, year (range)	72.5 (24-94)	72 (24-94)	80 (67-89)	0.126
Sex				1
Male, n (%)	33 (46)	28 (45)	5 (50)	
Female, n (%)	39 (54)	34 (55)	5 (50)	
Blood counts				
Hemoglobin, g/L, median (range)	89 (40-144)	92 (40-144)	81 (57-94)	0.009
WBC $\times 10^9$/L, median (range)	4.42 (1.04-38.25)	4.24 (1.04-38.25)	5.67 (1.99-11.62)	0.143
ANC $\times 10^9$/L, median (range)	2.25 (0.20-35.19)	2.30 (0.20-35.19) 140 (11-560)	2.33 (0.27-7.79)	0.558
Platelets $\times 10^9$/L, median (range)	145 (11-805)	7 (11.3)	195 (109-805)	0.025
BM ring sideroblast seen, n (%)	15 (21)		8 (80)	<0.001
WHO classification, n (%)				<0.001
MDS-SLD	21 (29.2)	20 (32.3)	1 (10)	
MDS-MLD	22 (30.6)	22 (35.5)	0 (0)	
MDS-RS	6 (8.3)	1 (1.6)	5 (50)	<0.001
MDS with isolated del(5q)	1 (1.4)	1 (1.6)	0 (0)	
MDS-EB-1	11 (15.3)	8 (12.9)	3 (30)	
MDS-EB-2	3 (4.2)	3 (4.8)	0 (0)	
MDS-U	6 (8.3)	5 (8.1)	1 (10)	
Transformation to AML	2 (2.8)	2 (3.2)	0 (0)	
Karyotype risk categories, n (%)				0.75
Very good	3 (4)	3 (4.8)	0 (0)	
Good	42 (58)	37 (59.7)	5 (50)	
Intermediate	20 (28)	16 (25.8)	4 (40)	
Poor	5 (7)	4 (6.5)	1 (10)	
Very poor	2 (3)	2 (3.2)	0 (0)	
IPSS-R risk classification, n (%)				0.13
Very low	5 (6.9)	5 (8.1)	0 (0)	
Low	29 (40.3)	27 (43.5)	2 (20)	
Intermediate	23 (32)	16 (25.8)	7 (70)	0.01
High	9 (12.5)	8 (12.9)	1 (10)	
Very high	6 (8.3)	6 (9.7)	0 (0)	

(47%) whereas 38 patients (53%) represented abnormal karyotypes (Figure 2). According to the IPSS cytogenetic risk groups, the patients with the *SF3B1* mutations were categorized into good karyotype risk (normal, n = 4; del (20q), n = 1), intermediate risk (+8, n = 1; del (7q), n = 1; add (18q), n = 1; del (13q), n = 1) and poor risk (inv (3), n = 1), respectively. To gather, we demonstrated the clinical and laboratory data of 72 MDS patients which were defined by their *SF3B1* mutation statuses.

The distribution of SF3B1 mutations in MDS patients

In this report, we could perform direct sequencing technique to investigate *SF3B1* mutation status in

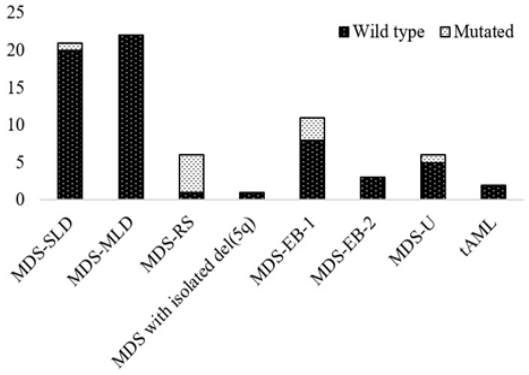

Figure 4. The Distribution of the *SF3B1* Mutations in Different Subtypes of MDS

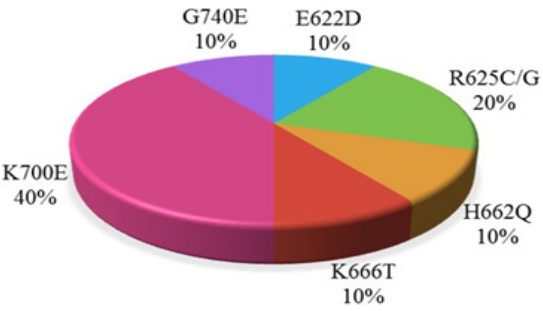

Figure 5. Types and Frequencies of *SF3B1* Mutations in Patients with MDS (n=10).

Table 3. The Distribution of *SF3B1* Mutations in 72 MDS Patients and the Resulting Amino Acid Changes

Gene	Exon	Base change	Effect	Amino acid change	Frequency (%)
SF3B1	14	37281 G>T	Missense	E622D	1/72 (1.39%)
SF3B1	14	37288 C>T/G	Missense	R625C/G	2/72 (2.78%)
SF3B1	14	37401 C>G	Missense	H662Q	1/72 (1.39%)
SF3B1	14	37412 A>C	Missense	K666T	1/72 (1.39%)
SF3B1	15	37938 A>G	Missense	K700E	4/72 (5.56%)
SF3B1	15	38059 G>A	Missense	G740E	1/72 (1.39%)

Table 4. The Distribution of *SF3B1* Mutation in Myelodysplastic Syndrome from Different Countries

Study	MDS / Sample size (%)	MDS with RS / Sample size (%)	Country
This study	10/72 (13.9%)	8/15 (53%)	Thailand
Cui et al., 2012 (Cui et al., 2012)	NA	55/104 (53%)	China
Lin et.al., 2014 (Lin et al., 2014)	48/ 479 ((10%)	21/34 (61.8%)	Taiwan
Papaemmanuil et al., 2011 (Papaemmanuil et al., 2011)	72/354 (20.3%)	53/82 (65%)	United Kingdom
Malcovati et al., 2011 (Malcovati et al., 2011)	150/533 (28.1%)	NA	Italy
Damm et al., 2012 (Damm et al., 2012a)	37/221 (16.4%)	NA	France
Damm et al., 2012 (Damm et al., 2012b)	47/317 (14.8%)	28/50 (56%)	Germany, France
Thol et al, 2012 (Thol et al., 2012)	28/193(14.5%)	NA	Germany
Malcovati et al., 2015 (Malcovati et al., 2015)	NA	151/243 (62.1%)	Italy, Sweden, Denmark
Patnaik et al., 2012 (Patnaik et al., 2012b)	NA	53/107 (49.5%)	USA
Donaires et.al., 2016 (Donaires et al., 2016)	6/91 (6.59%)	NA	Brazil

genomic DNA isolated from bone marrow samples of MDS patients (Figure 3). We could detect 10 heterozygous point mutations of *SF3B1* in 10 out of 72 (14%) MDS patients. The most frequent *SF3B1* mutation was K700E (4 of 10) followed with R625C/G (2 of 10), E622D (1 of 10), H662Q (1 of 10), K666T (1 of 10), and G740E (1 of 10), respectively (Table 3, Figure 4, and Figure 5). Interestingly, all identified mutations were recognized as missense mutation which were clustered in exons 14 and 15 of *SF3B1* coding sequence. Furthermore, *SF3B1* mutations were predominantly observed in 5 out of 6 (83.3%) in patients with MDS-RS ($p < 0.001$). Additionally, *SF3B1* mutations were positive in 3 patients who presented ring sideroblast and were classified as MDS-EB-1. Moreover, the mutations were also detected in 1 case of MDS-SLD and 1 case of MDS-U. Nevertheless, we could not detect *SF3B1* mutation in MDS patients with isolated del(5q), MDS-EB-2, and secondary AML (Figure 4). To gather, we reported the distribution of *SF3B1* mutations in Thai MDS patients. *SF3B1* missense mutations (all heterozygous) were strictly located in exon 14 and 15. The data further highlighted that *SF3B1* is associated with the development of MDS with ring sideroblast.

Discussion

In the past few years, several studies reported that genetic mutations in genes encoding proteins critical for RNA splicing process are involved in the establishment of MDS and other hematological malignancies. Somatic mutations of *SF3B1*, a component of U2 RNA spliceosome have been reported to be associated with the initiation of MDS with ring sideroblast. More recently, the 2016 WHO classification of myeloid neoplasm and acute leukemia had included the molecular analysis of *SF3B1* mutations into the criteria for the diagnosis and classification of MDS (Arber et al., 2016). In this report, we established the PCR sequencing technique to detect the hotspot mutations of *SF3B1* (exon 13 to exon 16) in bone marrow specimens of 72 MDS patients with different WHO subtypes. Moreover, we described the clinical and laboratory characteristics of MDS patients regarding to their *SF3B1* mutation statuses. Similar to several studies (Jeromin et al., 2015; Malcovati et al., 2014; Papaemmanuil et al., 2011; Visconte et al., 2012; Yoshida et al., 2011; Zhu et al., 2016), we observed that *SF3B1* is predominantly mutated in MDS patients with ring sideroblast (83.3%). Moreover, we found that the overall frequency of *SF3B1* mutations was comparable to recent reports (Table 4). Additionally, we found that mutations of *SF3B1* are frequently occurred at the c-terminal heat domains (amino acid residues 622-781) which the data is consistent to previous reports (Malcovati et al., 2011; Papaemmanuil et al., 2011; Seo et al., 2014). In this report, K700E was the most common *SF3B1* mutation residue (40% of mutated cases) and additional hotspot mutations were following R625C/G (20%), E622D (10%), H662Q (10%), K666T (10%), and G740E (10%), respectively. Although other hotspot mutations of *SF3B1* such as mutations at codon 623 and 663 were not detected in this report due to the limited in the number of studied population ($n = 72$), our data further suggested that *SF3B1* mutations was strongly associated with the development of MDS with ring sideroblast phenotype.

Analysing the clinical characteristic of patients at the time of mutation analysis, we found that patients with *SF3B1* mutations showed significantly lower in

haemoglobin level and higher in platelet count when compared with patients without *SF3B1* mutations. However, we did not find significantly different in several tested parameters including age, sex, and leukocyte count in both patients with and without *SF3B1* mutations. Similar to previous reports, patients with *SF3B1* were clustered in the good (5/10) and intermediate (4/10) cytogenetic risk group. Additionally, the majority of *SF3B1* mutated cases were categorised as IPSS-R intermediate risk group. Our data further confirmed that *SF3B1* mutations were associated with a favourable prognosis phenotypes in MDS patients (Malcovati et al., 2014; Malcovati et al., 2011; Papaemmanuil et al., 2011; Patnaik et al., 2012b; Patnaik and Tefferi, 2015; Patnaik and Tefferi, 2017; Seo et al., 2014). Although several research groups demonstrated that *SF3B1* mutation was associated with the independent favourable prognosis in MDS, there were few studies reported that *SF3B1* mutations were not resulting in different overall survival of patients (Damm et al., 2012a; Thol et al., 2012). The optimistic prognosis value of MDS patient with *SF3B1* may be interfered by several factors including the appropriated selection of survival analysis end points, and remarkably the present of additional/cooperating genetic mutations during disease progression [e.g., the co-mutation of DNM3TA(Bejar et al., 2012)]. Thus, this further highlights the clinical importance of the molecularly identification of *SF3B1* and other MDS/MPN associated mutations for disease classification, prognostication, and monitoring during the treatment.

In conclusion, we established PCR sequencing technique to analyse somatic mutations of *SF3B1* exon 13 to 16 in 72 Thai patients with different MDS subtypes. *SF3B1* mutations were predominantly occurred in MDS with ring sideroblast and categorised as good and intermediate MDS risk groups. This study further highlights the clinical important of *SF3B1* mutation analysis for the molecular classification of MDS.

Conflict of interest

The authors declare that they have no conflict of interest.

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