MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 5(4) pp. 192-196, April, 2017
Available online http://www.meritresearchjournals.org/mms/index.htm

Case Report

Co-occurrence of Oncogenic Driver Mutations (*SF3B1, RUNX1 and CSF3R*) with Distinct Prognostic Implication in a Lower Risk Myelodysplastic Syndrome Patient

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Abstract

Recurrent cytogenetic abnormalities are demonstrated in approximately fifty percent cases of Myelodysplastic syndromes (MDS) found as result of genomic instability accredited to the presence of oncogenic genetic mutations. Over the years the molecular basis of MDS has remained elusive but major breakthroughs have been made recently in elucidating the molecular pathogenesis of this entity by employing the sophisticated technology of next generation sequencing. We are reporting here a circumstantial account of a case of Myelodysplastic syndrome with ringed sideroblasts and multilineage dysplasia (MDS-RS-MLD) with normal karyotype and co-occurrence of oncogenic mutations involving *SF3B1*, *CSF3R* and *RUNX1,* deciphered through next generation sequencing. The reports pertinent to the association of *SF3B1* mutation to other driver mutations are relatively sparse. It most commonly exists in association with *DNMT3A* and the two together play an important role in evolution of MDS. *RUNX1* mutations are the second common mutations to exist in association with *SF3B1*. This case points not only towards the diagnostic importance of these somatic driver mutations but also demands for a more refined prognostic model with the integration of somatic driver mutations so that better prognostic groups can be assigned and better risk adapted treatment can be offered to individual MDS patients.

Key words: Myelodysplastic syndrome, Next Generation Sequencing, SF3B1, RUNX1, CSF3R mutation

INTRODUCTION

Myelodysplastic syndrome (MDS) is heterogeneous hematological entity specified by the presence of peripheral cytopenias and dysplasia in association with ineffective hematopoiesis and an increased propensity to evolve in to acute myeloid leukemia (AML) (Arber et al., 2016; Campo et al., 2011). Recurrent cytogenetic abnormalities are demonstrated in approximately fifty percent cases of MDS and are an important tool of establishing clonality and defining prognosis (Haase et al., 2007). These cytogenetic abnormalities are surmised to be the result of genomic instability accredited to the presence of oncogenic genetic mutations (Lindsley and Ebert, 2013). Presence of driver genetic mutations has been substantiated in ninety percent cases of MDS, of which sixty seven percent are found in patients with normal karyotype (Harada and Harada, 2015). Over the years the molecular basis of MDS has remained elusive but major breakthroughs have been made recently in elucidating the molecular pathogenesis of this entity by employing the sophisticated technology of next generation sequencing. Mutated genes implicated in the pathogenesis of MDS involve mRNA splicing machinery, DNA methylators, chromatin modifiers, transc-ription factors, signal transduction proteins and cohesion

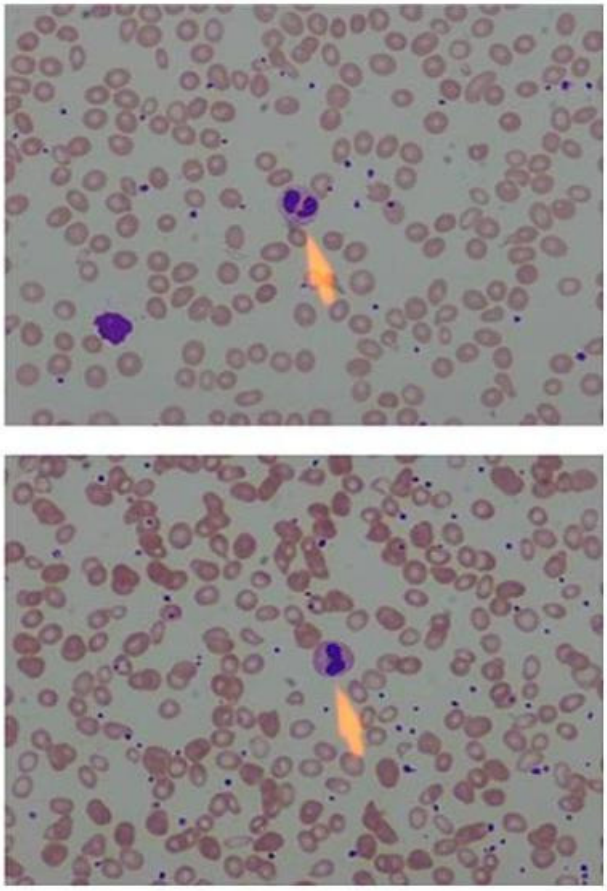

Figure 1. Peripheral smear illustrated bicytopenia with normochromia and macrocytosis, along with dysplastic hypolobated and hypogranular neutrophils

complex components. Some of these mutation play the role of somatic foundation mutations and others are considered to be subclonal (Cazzola et al., 2013; Kulasekararaj et al., 2013). *SF3B1*, a spliceosome gene mutation, has an uncertain prognostic significance (Lin et al., 2014). *RUNX1*, a transcription factor gene mutation, is considered to be an independent predictor of poor outcome (Bejar et al., 2012). *CSF3R* has strong phenotypic association with chronic neutrophilic leukemia (CNL) and deemed to be a foundational driver mutation for this entity (Cazzola et al., 2013). We are reporting here a circumstantial account of a case of Myelodysplastic syndrome with ringed sideroblasts and multilineage dysplasia (MDS-RS-MLD) with normal karyotype and co-occurrence of oncogenic mutations involving *SF3B1* and *RUNX1,* deciphered through next generation sequencing.

Case Report

A Forty-six years old female with no known comorbid presented to us with the concerns of weakness, low-grade fever, and undocumented weight loss for six months. On examination, she was vitally stable. Her general physical examination revealed pallor and rest of the systemic examination was unremarkable. Evaluation of drug history demonstrated the use of hematinics in appropriate doses for three months. There was no history of blood products transfusion.

Her complete blood count showed Hb of 9g/dl with MCV 106 fl, MCH 34 pg, and MCHC 32 g/dl, WBC of $2.8x10^9$/L with ANC of $0.9x10^9$/L, and platelet count $326x10^9$/L. Peripheral smear illustrated bicytopenia with normochromia and macrocytosis, along with dysplastic, hypolobated and hypogranular neutrophils (Figure 1). Her biochemistry panel turned out to be normal. Serum B12 and RBC folate levels were within normal limits and her chest X-ray and Ultrasound abdomen were also normal.

Bone marrow aspirate and trephine was then performed and exhibited significant dysplasia in all three lineages (Figure 2). Erythropoiesis demonstrated nuclear/cytoplasmic asynchrony, nuclear budding and binuclearity. Myelopoeisis was dysplastic with hypogranulation and hypolobation along with maturation

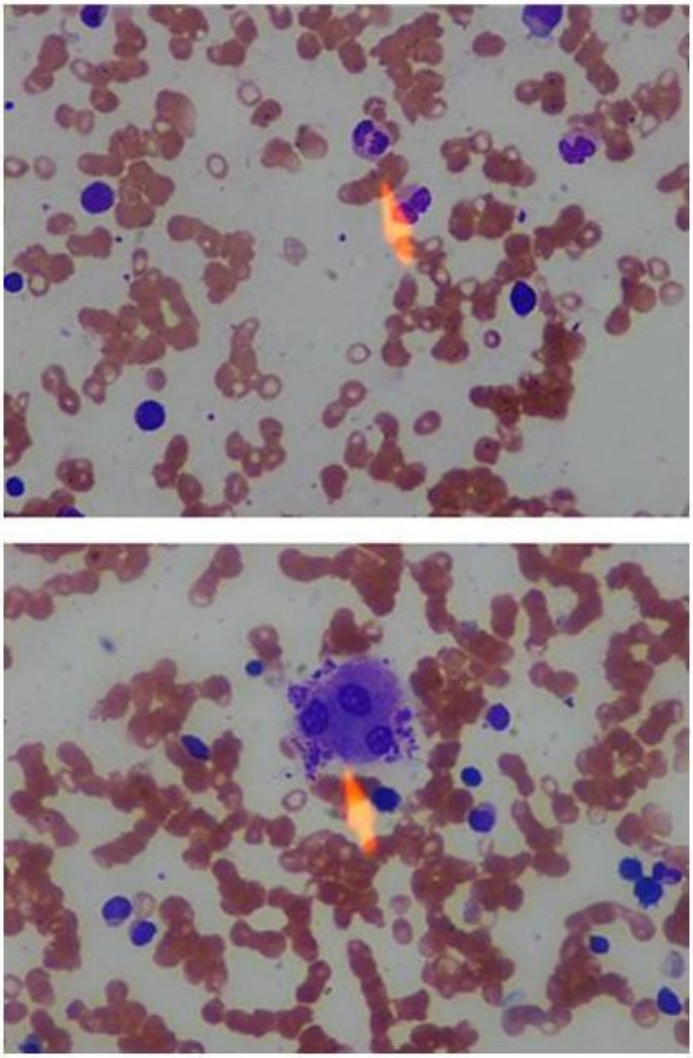

Figure 2. Erythropoiesis demonstrated nuclear/cytoplasmic asynchrony, nuclear budding and binuclearity. Myelopoeisis was dysplastic with hypogranulation and hypolobation along with maturation and differentiation. Megakaryocytes were adequate but were dysplastic and revealed multinuclearity and hypolobation.

and differentiation. Megakaryocytes were adequate but were dysplastic and revealed multinuclearity and hypolobation. Blast cell count were 04 percent of the total mononuclear cell population (500 cell differential). Iron staining revealed iron of grade two along with twenty percent ring sideroblasts (Figure 3). Reticulin stain showed MF-1. On the basis of above mentioned findings, diagnosis of Myelodysplastic syndrome with ringed sideroblasts and multilineage dysplasia was established. Bone marrow cytogenetics was performed and it revealed normal female karyotype (46XX).

This was then followed by next generation sequencing to unravel the presence of any somatic driver mutations. The DNA was extracted from the peripheral blood sample of the patient using a QIAamp DNA Blood Mini Kit (Qiagen) following the manufacturer's instructions. Research protocol was approved by the Institutional Review Board (ERC/IRB) and conformed to the tenets of the Declaration of Helsinki. Written informed consent was obtained from patient.

The myeloid sequencing panel of 54 genes (complete coding exons of 15 genes and exonic hotspots of 39 genes) was sequenced. The panel focuses on ~141 kb of genomic content consisting of ~250bp in length with the medium coverage of the sample was >95% of amplicons at >500x coverage. Amplicon libraries were prepared by TruSight myeloid sequencing panel (Illumina, CA) and paired-end sequencing runs were performed on a MiSeq (Illumina) genome sequencer. Data analysis alignment was performed on-instrument MiSeq Reporter software.

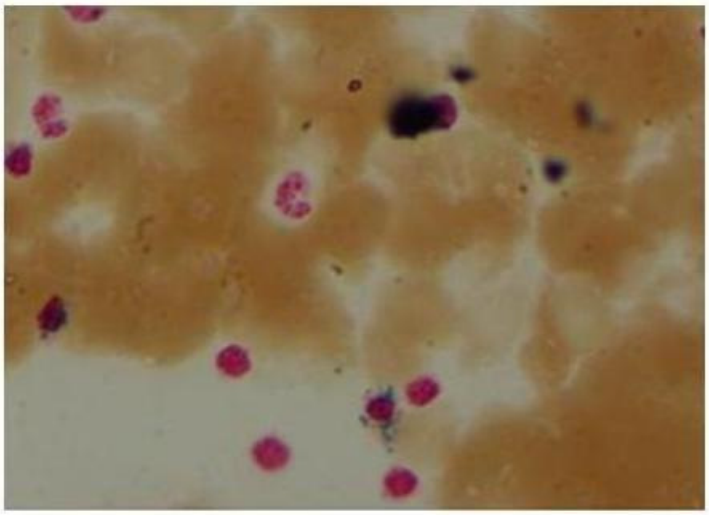

Figure 3. Iron stain illustrating ringed sideroblasts

The mutations identified as pathogenic were confirmed using the Sanger method following the standard protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems®). Genomic analysis was done using variant studio software v2.2. The medium coverage of the sample was >95% of amplicons at >500x coverage. Several databases, such as dbSNP, COSMIC and Ensemble were used to report mutations and search variants. We identified two reported missense mutation c.1997A>C, p.K666T (COSM110698) in *SF3B1* gene found to be deleterious (Score-0.01) on SIFT and probably damaging effect on Polyphen (Score-0.937), c.2503G>A, p.E835K (COSM4172010) in *CSF3R* gene with probably damaging effect on polyphen (score-0.993) along with one insertion mutation c.67insA, p.S226KfsTer2 in *RUNX1* gene have diseases causing status on Mutation Taster.

The patient was classified as IPSS Intermediate -1 risk group with the calculated score of 0.5 and IPSS-R low risk group with the computed score of 3. Her serum Erythropoietin level was >200 iu/ml. As per her lower risk group and current recommendations the patient is on injection Erythropoietin 40,000IU subcutaneously once per week along with Vitamin B_6 and she is maintaining her counts well without the need of blood products transfusion and has been on regular follow up every month for last 12 months.

DISCUSSION

Distinct insight has been acquired over the recent years about the molecular pathogenesis of MDS. Next generation sequencing and mass spectrometry based genotyping has made it possible to screen the mutational hotspots. Current literature implicit approximately 50-60 genes as myelodysplasia driver genes (Walter et al., 2012). Genotype – phenotype association has also been elucidated for various driver mutations (Harada and Harada, 2015).

NGS panel of our patient revealed *SF3B1*, *RUNX1*and *CSF3B* mutations. *SF3B1* is believed to be a foundational mutation. It creates a dominant clone of cellsthat gradually repopulates whole of the hematopoietic tissue. Over the course of disease due to acquiesced genomic instability a myriad of further subclonal mutations, like *RUNX1, ASXL1 or EZH2*, are fostered. Various subclones are generated in this manner further vitiating the differentiation, hampering the maturation, and causing disease advancement. The percentage of blasts gradually progresses, eventually evolving in to overt AML (Cazzola et al., 2013; Lin et al., 2014; Papaemmanuil et al., 2013). *CSF3R* is considered to be the driver mutation of CNL. The mutation exists in ninety percent cases of CNL and forty percent cases of atypical chronic myeloid leukemia (aCML). Its role has also been implicated in the progression of severe congenital neutropenia to AML/MDS.

SF3B1 mutations affect about 20-30 percent of the MDS cases and have a clear cut causal link with the presence of ring sideroblasts (Cazzola et al., 2013; Lin et al., 2014). WHO 2016 classifies MDS patients with more than five percent ring sideroblasts and *SF3B1* mutation as MDS-RS (Skokowa et al., 2014). Its prognostic implication remains ambiguous though most of the literature search claims that it imparts a better overall and leukemia free survival. *RUNX1* is a transcription factor gene and alterations in this gene found in approximately ten percent of MDS cases. It is considered to be a subclonal mutation and has a high mutational frequency with chronic myelomonocytic leukemia (CMML). Its occurrence implies advanced disease and poor patient

outcome in all myeloid neoplasms (Cazzola et al., 2013).

The reports pertinent to the association of *SF3B1* mutation to other driver mutations are relatively sparse. It most commonly exists in association with *DNMT3A* and the two together plays an important role in evolution of MDS. *RUNX1*mutations are the second common mutations to exist in association with *SF3B1*. In a study, three patients with *SF3B1* mutation whose disease progressed and were serially analyzed, found to acquire *RUNX1* mutation. Of the three patients, the first patient was labeled as RAEBT and the disease transformed to AML after running a stable course for eighteen months. Mutational identification reveals the acquisition of *RUNX1*. Similarly, for the second patient, diagnosed as RARS, the disease evolved in to AML after seven months. Sequential mutational analysis demonstrated *RUNX1* mutation. For the third RARS patient, the disease transformed to CMML after acquiring *RUNX1* mutation (Lin et al., 2014). An extensive literature search was unable to demonstrate the association of these three mutations, although the cooperation of *CSF3B* and *RUNX1* in driving leukemogenesis in congenital neutropenia is well established (Skokowa et al., 2014).

IPSS-R stratifies our patient in lower risk group that predicts better overall survival and longer acute leukemia free survival. But the presence of *RUNX1* mutation shifts the prognosis towards the opposite direction as the literature search clearly demonstrates that *RUNX1* is an independent predictor of poor outcome (Bejar et al., 2012). This knowledge prompts us to treat the patient as one with high risk of disease transformation and poor overall survival.

This case points not only towards the diagnostic importance of these somatic driver mutations but also demands for a more refined prognostic model with the integration of somatic driver mutations so that better prognostic groups can be assigned and better risk adapted treatment can be offered to individual MDS patients.

Conflicts of Interest

The authors have no conflicts of interest relevant to this article.

Funding

None

Author's Contribution

AJ and NI contributed in literature search and manuscript writing. SS and SA contributed in genomic lab work. TS contributed in editing and critically reviewed the manuscript.

ACKNOWLEDGMENTS

We acknowledge our research fellow for her immense support.

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