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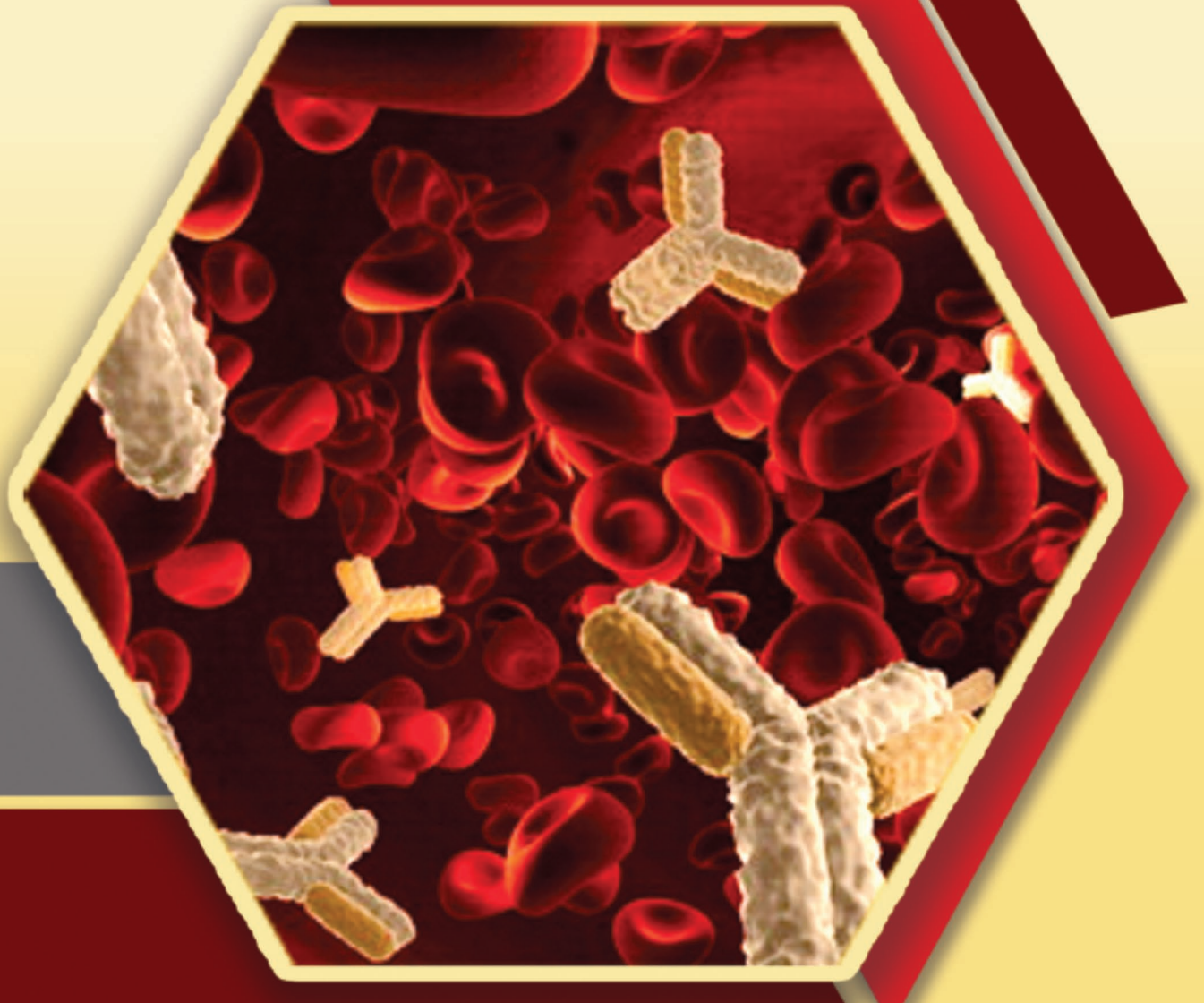

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Spliceosomal Mutations in Myelodysplastic Syndromes and Its Therapeutic Implications-An Overview

Purvi Mohanty

Genomic instability as a result of mutation and recombination is the hallmark of leukemogenic transformation. Recently, alternative splicing has been shown to be crucial for genomic maintainance. Alternative Splicing is a vital physiologic process in eukaryotic gene expression that allows for expression of multiple mRNA isoforms from an individual gene, encoding protein isoforms of different functionality. It affects the process of differentiation, human development, and its disruption underlies many human diseases including cancer (1-2). Eukaryotic genes are transcribed as premature messenger RNA (pre-mRNA), consisting of exons interrupted by non-coding introns. The removal of introns and exon joining is mediated by the process of pre-mRNA splicing which primarily occurs co-transcriptionally and is carried out in the nucleus by the spliceosome, a macromolecular machine consisting of five small nuclear ribonucleoproteins (U1, U2, U4, U5 and U6 snRNAs) and > 150 proteins (3).

Somatic mutations of spliceosomal genes were unknown until recently described to disrupt several processes downstream, because an altered spliceosome may cause abnormal transcription as well as altered splicing outcomes such as exon skipping, intron retention, and cryptic splice site activation with truncated (or elongated) exon, of thousands of genes (4). Through these mechanisms, somatic mutations of the RNA splicing machinery can play a role in the pathogenesis of human cancers, particularly in the pathophysiology of hematologic malignancies. Spliceosomal genes have been described as the most common targets of somatic point mutations in MDS, suggesting that dysregulated splicing may constitute a common theme linking the heterogenous disorder of MDS. Here in this review, we summarize the current understanding of the molecular machinery involved in splicing, discuss the spliceosomal alterations that arise in MDS, how they contribute to its pathogenesis with the emergence of a new leukemogenic pathway and then discuss the possibility for therapeutic intervention in MDS.

The Intricate Process of Pre-mRNA Splicing

Post-transcriptional processing of primary transcripts to form mature mRNA species involves the removal of intronic sequences by the spliceosome and this intricate process is called Pre-mRNA splicing. Pre-mRNA splicing is catalyzed by the spliceosome, a macromolecule composed of 5 small nuclear RNAs (snRNAs) associated with proteins to form particles called small nuclear ribonucleoproteins (snRNPs) and it involves the sequential binding and release of snRNPs and numerous auxiliary protein factors as well as the formation and disruption of RNA–RNA, protein–RNA and protein–protein interactions (5).Pre-mRNA splicing and its regulation requires a

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complex array of cis elements, embedded in pre-mRNAs as well as trans factors that bind to these elements. The recognition of the proper splice sites is critical for pre-mRNA splicing and a key point for the regulation of gene expression (3).

In most eukaryotes, including humans, there are two classes of introns: the common U2 type, representing more than 99% of human introns, and the rare U12 type, with the two differing in their consensus splice-site sequences (6). The "major" or U2-type spliceosome, which consists of the U1, U2, U4, U5, and U6 snRNPs, catalyzes the vast majority of transcript splicing events, whereas the "minor" or U12-type spliceosome utilizes the U12 snRNP to mediate splicing of approximately 800 specific transcripts (5,6). The process of splicing is initiated by the spliceosome assembly phase, which includes the sequential formation of three complexes: an early ATP independent "E" complex, an "A" complex and a "B" complex. "E" complex formation results from the binding of a U1 snRNP to the 5' splice site, the binding of splicing factor 1 (SF1) to the branch point and of the U2 auxiliary factor (U2AF) heterodimer to the polypyrimidine tract and 3' terminal AG (Fig.1). The U2AF protein is a heterodimer consisting of a U2AF1 subunit (also called U2AF35) and a U2AF2 (or U2AF65) subunit, which mediate binding to the 3'splice site and the polypyrimidine tract, respectively (7). SR proteins facilitate splice site recognition by binding to enhancer regulatory sequences (8). After the 5′ and 3′ splice sites are recognized and exons are defined intron definition occurs by cross-intron interaction between U1 and U2snRNPs, to form a functional spliceosome. The E complex is converted into the ATP-dependent, pre-spliceosomal "A" complex after the replacement of SF1 by the U2 snRNP at the branch point bringing together the proximal and distal exons. ATP hydrolysis is required for splice site pairing, which locks splice sites into a splicing pattern after U2 snRNP binding to the branch site. U2 snRNP consists of a 12S RNA subunit and the SF3A and SF3B multiprotein complexes, with SF3B1 mediating binding to the intronic branchpoint sequence. After the formation the A complex, the U4/U6-U5 pre-assembled tri-snRNPs are recruited, generating the B complex which contains all snRNPs however, it is still catalytically inactive. Major compositional and conformational rearrangements take place resulting in the releasing of U1 and U4 snRNPs and U5 snRNA binding to the exon sequences near splice site to juxtapose the neighboring exons. At this stage, 5' end of U6 snRNA, replacing U1snRNA, base pairs with 5'ss by its conserved ACAGA box. In addition, extensive base pairing and structural rearrangements happen between U6 and U2 for subsequent catalytic activation; generate the activated spliceosome (the B activated complex). The activated spliceosome then carries out the first catalytic step of splicing, yielding the C complex which undergoes further rearrangements and then catalyzes the second step of splicing (9).

Regulation of Splicing

Traditionally, splicing has been thought to be regulated by splicing enhancers and silencers. These short, conserved RNA sequences are typically 10 nt in length, are located either in exons or introns, acting either isolated or in clusters, and stimulate (enhancers) or inhibit (silencers) the use of splice

sites through the specific binding of regulatory proteins such as SR proteins (serine/arginine rich proteins) or heterogeneous nuclear ribonucleoproteins (hnRNPs) for correct recognition of exons and modulation of splicing outcomes (10). Depending on the position and function of the cis-regulatory elements, they are divided into four categories: exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs) and intronic splicing silencers (ISSs). ESEs are usually bound by members of the SR (Ser–Arg) protein family (10). ISSs and ESSs are commonly bound by hnRNPs which have one or more RNA-binding domains and protein–protein interaction domains; ISE's however, are not well characterised (11).The cross-exon ommunication between the factors that bind to these elements allow the exon to be recognized as a unit(exon definition) prior to intron removal. Most, if not all, of the spliceosomal proteins involved in the removal of introns from the primary transcript also influence patterns of alternate splicing.SR proteins have important roles in facilitating splice site recognition. The SR proteins contain one or more RNA binding domains (Arg–Ser repeat-containing) that mediate their ability to interact with intronic and exonic splicing enhancers (ISEs and ESEs, respectively) present in many primary transcripts (8,9). Binding of SR proteins at these enhancer elements enables recruitment of the U1 snRNP and U2AF to the neighboring 5' and 3' splice sites, by binding to an ESE and directly interacting with protein targets, promoting the inclusion of that exon into the mature transcript. SR proteins also co-operate with other positive regulatory factors to form larger splicing enhancing complexes by interacting with other RS domain-containing proteins (11). However, this process is regulated by post-translational modification; methylation, acetylation, or phosphorylation of SR proteins alters their ability to bind ISEs and ESEs. Heterogeneous nuclear ribonucleoprotein complexes bind to intronic or exonic splicing silencer elements (ISSs and ESSs), blocking the recruitment of the U1 snRNP and U2AF by a mechanism that has not been fully defined (11). Most primary transcripts that have been studied contain multiple ISE/ESE or ISS/ESS elements; it is the overall balance between the activities at these two antagonistic classes of regulatory element that determines whether or not a particular exon will be included in the mature transcript. Inhibition of splice site recognition can be achieved in many ways. First, when splicing silencers are located close to splice sites or to splicing enhancers, inhibition can occur by sterically blocking the access of snRNPs or of positive regulatory factors (12).Splicing of individual pre-mRNAs is frequently controlled by combinatorial or competitive effects of both activators and inhibitors. The final decision of whether an alternative exon is included is determined by the concentration or activity of each type of regulator, often by SR proteins and hnRNPs (11).

Mutations of Spliceosomal Genes in Haematological Malignancies

The targets for the majority of spliceosome-associated mutations in MDS are components of the “E” and “A” splicing complexes (13). Whole genome or exome sequencing of tumor and matched normal tissues in myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), and chronic lymphocytic leukemia (CLL) patients, along with

targeted sequencing of candidate genes, identified a high percentage of recurrent somatic mutations in genes encoding spliceosome components (14). Multiple independent somatic mutations were identified in the genes encoding U2AF1, ZRSR2, SF3B1, and SRSF2 involved in recognition and binding of the 3′ splice site. U2AF1 is exclusively required for splicing of U2 introns, whereas ZRSR2 is required for splicing of both U2 and U12 introns. SF3B1 and SRSF2 are expected to participate in the splicing of both U2 and U12 introns. SF3B1 mutations strongly correlate with refractory anemia with ring sideroblasts (RARS), a distinct type of low-risk MDS (14).The phenotypic associations of these mutations in MDS include: SF3B1 mutations which occur in earlystage MDS and indicate better prognosis; conversely,

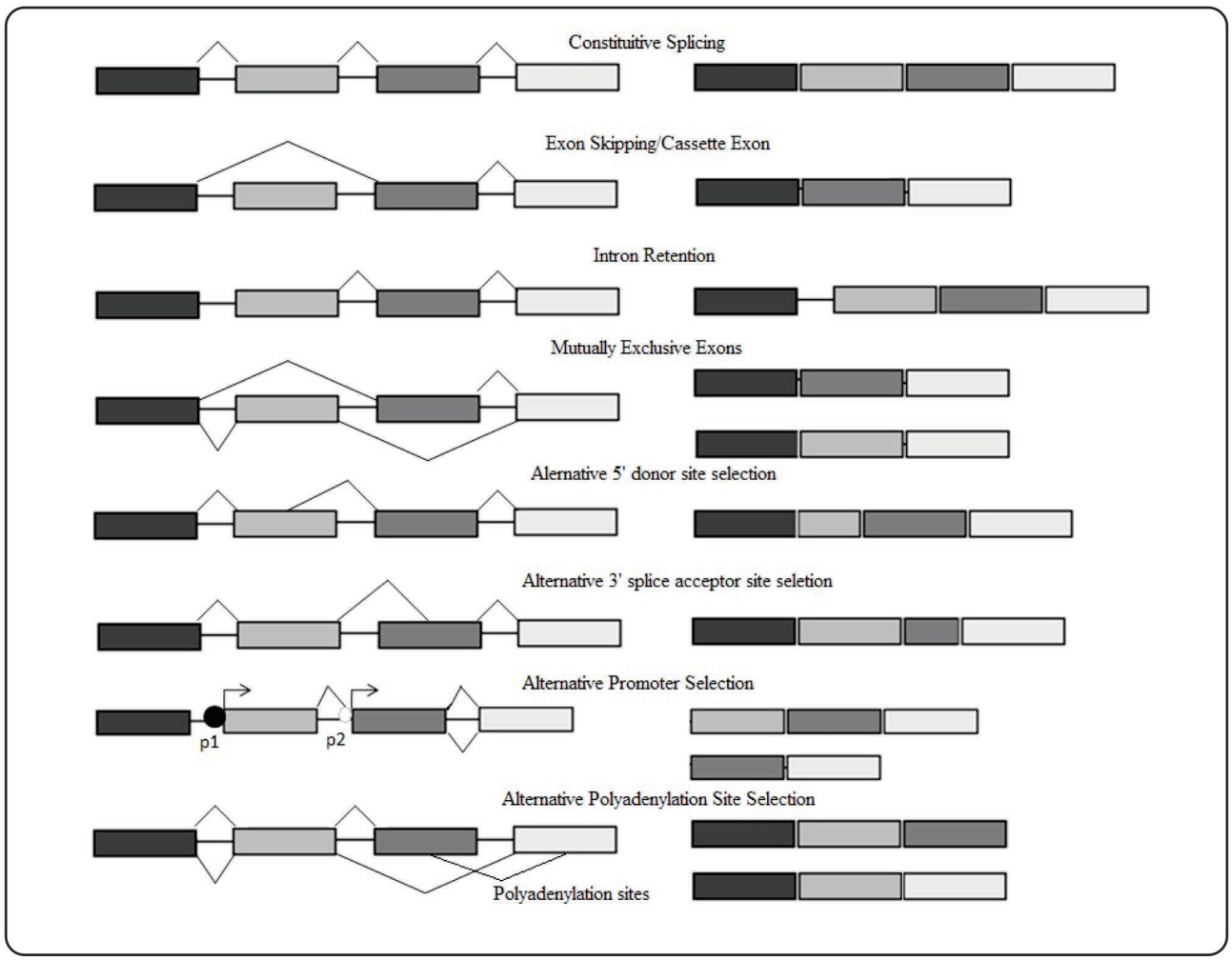

Figure 1: *Pre-mRNA splicing mechanism by the U2-type spliceosome: A pre-mRNA contains conserved sequences in intron and exon which are necessary for splicing. The 5'splicing site (5ss) and the 3' splicing site (3ss) define the intron and participate in the assembly of the spliceosome together with the branch site (A). Exonic splicing enhancers (ESEs), Exonic splicing silencers (ESSs) are bound by trans regulators, such as members of SR and hnRNP protein family, to either enhance or repress splicing via interacting with basal splicing machinery. Pre-mRNA splicing occurs via the formation of E, A, B and C complexes through several RNA-RNA, RNA- protein interactions.*

U2AF1 and SFRS2 mutations are frequently detected in advanced MDS and correlate with poor prognosis and shorter survival (15). Most of these mutations are simple amino acid substitutions, although small in-frame deletions within SRSF2 have been noted in patients with myelofibrosis or de novo MDS (13). Most disease-associated mutations can be localized to one of the functionally important domains in SF3B1, U2AF1, or SRSF2 (Fig.2). The most commonly mutated spliceosomal factor genes in MDS have been described below.

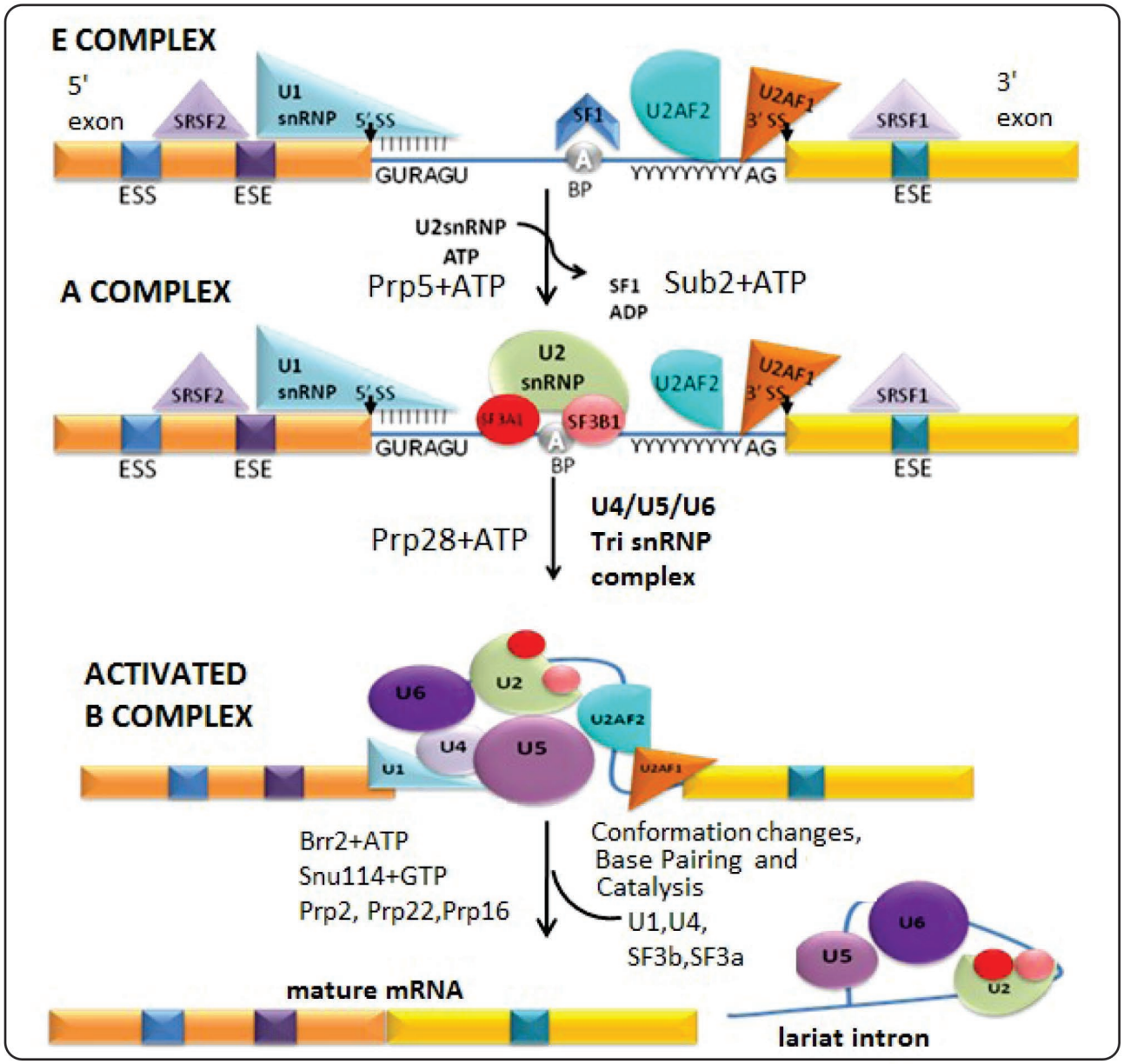

Figure 2 : *The frequent mutations of splicing factor genes in Myelodysplastic Syndromes. The upper panel depicts hotspot SF3B1 mutations in myeloid cancers particularly MDS as segregated WHO subgroup wise and the lower panel describes common mutations of SF3B1 in non-myeloid cancers. The frequent mutations of U2AF1 in Zn1/2(zinc finger domains) and SRSF2 in MDS are shown.RRM (RNA recognition motif).*

SF3B1

The most commonly mutated spliceosomal factor gene is located on chromosome 2q33.1. This gene codes for subunit 1 of the splicing factor 3b protein complex (SF3B1), which is involved in the early stages of spliceosomal assembly (16). The SF3B1 complex is part of the functional form of the U2 snRNP that binds to the branch site near the 3′ end of introns and helps to specify the site of splicing. It cross-links to a 25-nucleotide region in the pre-mRNA located immediately upstream of the intronic branch site (16). Mutation of the Splicing Factor 3B, subunit 1 (SF3B1) gene occurs in over 70% of patients with ring sideroblasts (RARS) however, how it affects clonal development or the formation of ring sideroblasts is still unclear . In SF3B1, most mutations are associated with one of the 22 HEAT Huntingtin, Elongation factor 3, protein phosphatase 2A and targets of rapamycin 1 repeats present in this polypeptide which mediate its interaction with other proteins (13). These mutations cluster around residues 625, 666, and 700 present in exons 14, 15 with K700E substitution occurring most frequently (14). In MDS patients harboring SF3B1 mutations; higher platelet count, increased ring sideroblasts, fewer cytopenias, lower blast count and longer event-free survival have been associated (17). Mutations in this gene have been identified in chronic lymphocytic leukaemia (CLL), uveal melanoma, pancreatic cancer, and at lower frequencies in breast cancer and de novo AML. These mutations have been shown to contribute to disease by deregulating specific target genes rather than generally impairing splicing (18). SF3B1 mutations are associated with diverse alternative splicing events, including alternative terminal exon usage, intron retention, and cryptic splicing within exons of both protein coding and noncoding genes. In CLL, SF3B1 mutations are associated with alternative splicing at the 3′ ends of genes to generate truncated variants of the vitamin C transporter SLC23A2, the T-cell regulator TC1RG1, and the forkhead transcription factor FOXP1 (18). Knockdown of SF3B1 in haematopoietic cell lines results in impaired cell growth, deregulated global gene expression and aberrant splicing. RNA-Seq studies of MDS patients showed aberrant plicing of genes involved in several critical cellular pathways, including cell cycle, mitochondrial function and RNA processing (18).

U2AF1

U2AF1 (U2AF35) gene located on chromosome 21q22; frequently mutated in MDS (14,19), belongs to the SR family genes and encodes the small subunit of U2 auxiliary factor complex required for recruitment of U2 snRNP to the pre-mRNA branch site of the intron (20). U2AF1 forms a heterodimer with U2AF2 (U2AF65) resulting in constitutive and regulated RNA splicing by directly mediating interactions between the U2AF2 protein and other splicing regulators such as SRSF1 and SRSF2. U2AF1 binds the 3′ AG splice acceptor dinucleotide of the pre-mRNA target intron and U2AF2 binds the adjacent polypyrimidine tract . U2AF1 mutations are mutually exclusive; consistently affecting S34 and Q157 residues within the first and second CCCH zinc fingers of the protein. U2AF1 were associated with ASXL1 and DNMT3A mutations, genes relevant for

epigenetic regulation which is especially intriguing because chromatin and histone modifications have been shown to play a significant role in pre-mRNA splicing. These mutations have been reported at frequencies of upto 8.7% in proliferative phenotypes, including MDS/MPN and high-risk MDS, associated with leukemic evolution and poor prognosis . It is also identified in patients with isolated −20/20q- at a higher frequency (19). Early studies using gene reporter assays identified that overexpression of mutant U2AF1 in HeLa cells resulted in dysfunctional splicing marked by frequent inclusion of premature termination codons and intron retention, while another early study reported ncreased exon skipping in a minigene assay following mutant U2AF1 expression in 293T cells, as well as increased cryptic splice site usage in the FMR1 gene in MDS samples. Recently, it has been shown that U2AF1 mutations cause highly specific alterations in 3′ splice site recognition in myeloid neoplasms giving rise to different alterations in splice site preference and largely contributing to distinct downstream splicing events (21). These mutations have been suggested to cause both alteration/gain of function and loss of function . It has been reported to cause differential splicing of hundreds of tumor-associated genes, affecting biological pathways such as cell cycle progression (CEP164, EHMT1 and WAC), RNA processing (PTBP1, STRAP, PPWD1, PABPC4, and UPF3B), DNA methylation (DNMT3B), X chromosome inactivation (H2AFY), the DNA damage response (ATR, FANCA), and apoptosis (CASP8) implying they may have a role in myeloid leukemogenesis (22).

SRSF2

These genes are located on chromosome 17q25.2 is important for splice-site selection, spliceosome assembly, and both constitutive and alternative splicing (23). This protein belongs to the SR splicing regulatory factor family containing an RNA recognition motif (RRM) for binding RNA and an RS domain for binding other proteins. The RS domain is rich in serine and arginine residues and facilitates interaction between different SR splicing factors. These proteins bind to splicing regulatory sequence elements in pre-mRNA transcripts and to components of the spliceosome, and can either activate or repress splicing depending on the location of the pre-mRNA binding site (23). The proteins' ability to activate splicing is regulated by phosphorylation and interactions with other splicing factor associated proteins. SRSF2 mutations are stable during disease progression. Pro95 is the common mutation in SRSF2 affecting its binding to its target RNA's. SRSF2 plays a role in preventing exon skipping, ensuring the accuracy of splicing and regulating alternative splicing. Recently, P95H missense mutation and P95 to R102 in-frame 8 amino-acid deletion caused significant changes in alternative splicing of genes involved in cancer development and apoptosis (24). Also, gene knockout studies have shown that SRSF2 is essential for the functional integrity of the hematopoietic system and that these are likely involved in the pathogenesis of MDS (24). SRSF2 mutations though particularly associated with CMML it has been identified in different subtypes of MDS and were not associated with a specific IPSS risk profile or cytogenetic aberration. Also, they were associated with mutations in RUNX1, a gene coding for a transcription factor, as well as IDH1, a gene coding for an enzyme of the citric acid cycle.

ZRSR2

This gene is located on chromosome Xp22.2. associates with the U2AF heterodimer, which is required for the recognition of a functional 3′ splice site in pre-mRNA splicing, and thus may play a role in network interactions during spliceosome assembly. ZRSR2 interacts with the 3′-splice site of U2- and U12-dependent pre-mRNAs and promotes different steps in U2- and U12-dependent intron splicing (25). There are no mutational hotspots in ZRSR2, with alterations occurring throughout the protein most of which are amino acid substitutions that affect the RBD or distal zinc finger motifs, although many nonsense or frameshift mutations that result in protein truncation have been identified (13). The prognostic impact of mutations in this gene is not clear. Recently, shRNA-mediated knockdown of ZRSR2 has shown impaired splicing of the U12-type introns and RNA-sequencing of MDS bone marrow reveals that loss of ZRSR2 activity causes increased mis-splicing. These splicing defects involve retention of the U12-type introns, while splicing of the U2-type introns remain mostly unaffected. ZRSR2-deficient cells also exhibit reduced proliferation potential and distinct alterations in myeloid and erythroid differentiation in vitro (25).

Current Therapeutic Approaches in MDS

Currently several strategies involving the use of kinase inhibitors, deacetylase inhibitors, DNA methyltransferase inhibitors, novel agents altering cell metabolism, cytotoxic agents/cell cycle inhibitors, apoptotic inhibitors, immunomodulatory and immunosuppressive agents are under evaluation for MDS therapy. The efficacy of combination therapy through the use of azacitidine with lenalidomide, and azacitidine with vorinostat (a deacetylase inhibitor currently FDA approved for treatment of cutaneous T-cell lymphoma) is being explored for replacement of traditional therapeutic approaches in MDS as it has shown higher response rates (26). In higher risk MDS patients to improve their life expectancy in case of failure of traditional treatment approach, SGI110, a novel hypomethylating agent that is a dinucleotide of decitabine linked to guanosine has been shown to be effective by increasing resistance to degradation by cytidine deaminase (27). In lower-risk MDS, where the primary therapeutic goal is to improve hematopoiesis and reduce transfusion needs; the role of several kinase inhibitors and a novel agent ARRY-614, a dual p38 mitogen activated protein kinase/Tie2 kinase inhibitor which has shown hematologic improvement in patients for whom hypomethylating agents have failed yet who still meet IPSS criteria for lower risk disease; is being investigated (27). HSCT is a potentially curative approach however, for concerns about changes in quality of life or fear of adverse events make it unavailable to a wider variety of patients. Greater use of cord blood or haploidentical donors, use of novel reduced-intensity conditioning approaches in older patients and those with comorbidities, and graft manipulation with post-HSCT azacitidine or with specific immunotherapies all may increase the rate of HSCT use (28).

Spliceosome inhibitors and splice factor mutations

A targeted therapy refers to the new generation of cancer drugs that are designed to interfere with a specific molecular target that has a critical role in tumor growth. Two products derived from nature have been shown to interfere with the spliceosome, a molecule named FR901464, is a drug isolated from the fermentation broth of a strain of Pseudomonas sp. which was isolated from a sample of Japanese soil. Pladienolide B is a drug isolated from a strain of Streptomyces platensis. Both of these compounds interact and disrupt the function of the spliceosome by targeting and modulating SF3B1 (29). The interference of SF3B1 by these two natural products causes cell growth to stall in the G1 and the G2/M cell cycles of growth. Despite this ability, both of these compounds make poor candidates for treatment options the reason being, both compounds are chemically complex. Sudemycins are structurally less complex than their natural analogues, containing fewer stereocenters having better stability and are not degraded within biological fiuids (29). Sudemycins also have the same effects as the natural products and inhibited splicing, arrested cell growth in the G1 and G2/M phases, and modulated the function of SF3B1 (29). Treatment with Sudemycin in cell lines caused an accumulation of aberrantly spliced gene products. Sudemycin's effect on splicing makes it a good candidate for a potential targeted cancer therapy. Inhibition of splicing with spliceostatin A, a compound that targets the SF3B1 protein, or knock down of SF3B1 expression has been shown to alter the fidelity of branch site recognition by U2 snRNP, leading to specific alterations in the alternative splicing patterns of many but not all genes (29). Spliceostatin A and similar compounds inhibit cell proliferation, particularly of transformed cell lines, suggesting that modulation of SF3B1 activity by mutations could be oncogenic while also providing a target for therapy (30).

Experience at NIIH

MDS are clinically heterogeneous clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis that causes one or mare peripheral blood cytopenia and risk of progression to acute myeloid leukemia (AML). We have been working on this rare hematological malignant disease since more than 15 years with collaboration of Department of Hematology, KEM hospital to understand molecular pathology of MDS in India. Our study showed that the young patients (>50 years) are more affected than more than 50 years age group. The cytogenetic study with the combination of conventional cytogenetic (GTG banding karyotyping) and fluorescent in situ hybridization (FISH) have identified chromosomal aberration in 42% of the MDS patients. Our previous study showed copy number changes in 7 and 1 chromosome region. Recently our study identified copy number variation in EPO (7q22)and SEC 16 (7p11) genes. Molecular study using NGS in large cohort showed high frequency of mutation in spliceosomal genes (SF3B1, U2AF1, SRSF2) compared to epigenetic regulating genes (ASXI1, TET2, IDH2, DNMT3, Tp53) (fig 3,4,5). The advantage of NGS is that it identifies multiple mutations in MDS patients, which helps in understanding the prognosis of the disease. The gene expression of MDS patients with spliceosomal mutations were studied using microarray. Microarray data showed significant upregulation of genes related to chemokine signaling pathway, JAK–STAT pathway, CML pathway and AML pathway. Genes involved in signaling by GPCR were found to be significantly down regulated.

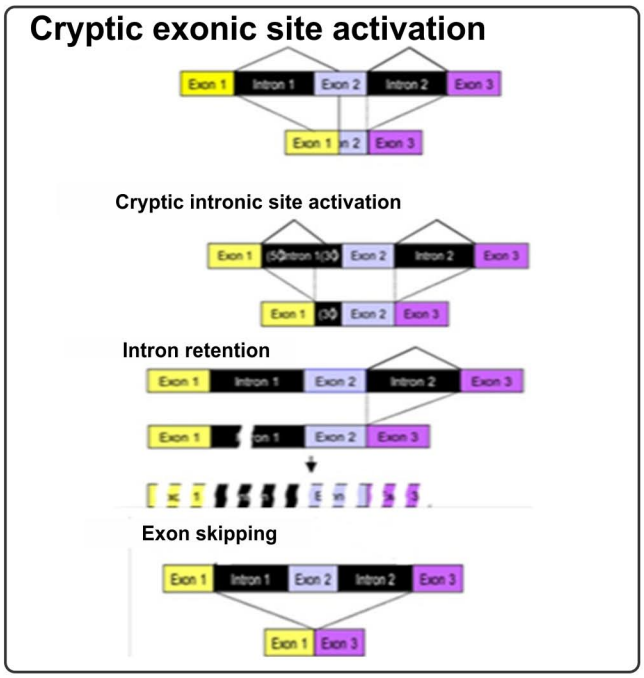

Figure 3 : Normal mechanism of splicing

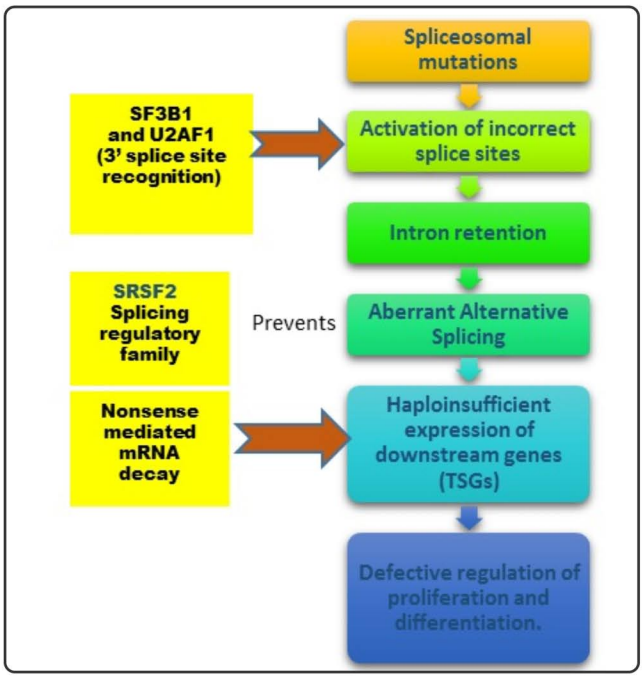

Figure 4 : *Consequences of splice mutations*

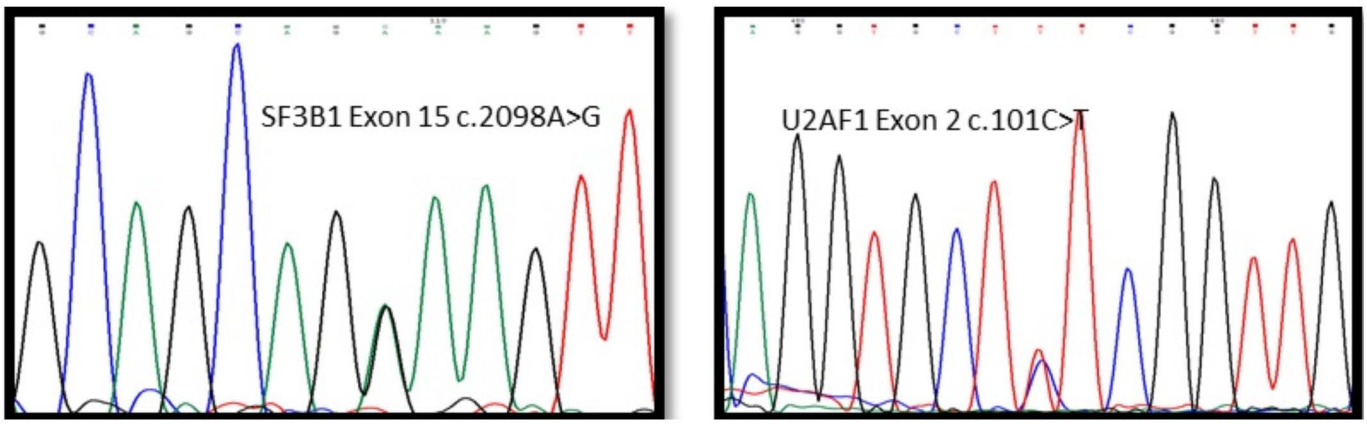

Figure 5 : *Chromatogram of showing mutations in splice factor genes SF3B1 and U2AF1*

Conclusion

Several questions regarding the biological consequences of these novel recurrent mutations and their suitability as targets for novel therapy remain incompletely answered. Further studies elucidating the phenotype associated with these recurrent point mutations and the gene expression pathways affected may enhance the understanding of the repercussions of spliceosomal alterations on neoplastic transformation as well as pave way for therapeutic intervention through novel mechanisms. However, until we decipher the rationale behind the complex clonal heterogeneity in MDS; only through innovations in stem cell therapy can we breakthrough in MDS therapy.

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माइलोडिस्प्लास्टिक सिंड्रोम (M.D.S.) में स्प्लाइसोसोमल उत्परिवर्तन एवं उनका चिकित्सकीय महत्वः एक अवलोकन

श्री सोमप्रकाश धनगर

सामान्यतः युकेरियोटिक कोशिकाओं में Pre-mRNA स्प्लाइसींग जीन एक्सप्रेसन हेतु एक महत्वपुर्णप्रकिया है। तथा द्वितीयक स्प्लाइसींग प्रोटीयोसोम विविधता की मुख्य कुंजीहै। चिकित्सा उपचार के क्षेत्र में स्प्लाइसोसोमल मशीनरीउपचारकीदृष्टिसे एक नगण्य केन्द्र रहा है। हाल ही मे M.D.S. पर अनुसंधान के दोरान इसकी चिकित्सा में स्प्लाइसोसोमल मशीनरी के ट्रान्स एक्टींग एलीमेण्ट में उत्पन्न होनेवाले उत्परिवर्तन एवं इनके पश्चात होनेवाले रक्त संवधी कर्क रोगों के बारे में जानकारी प्राप्त हुई है। तथा इन एलीमेण्टस में से मुख्यतः SF3B1, U2AF35 एवं SRSF2 जीन्स की रक्त संबधी कर्क रोगों मेंउच्च उत्परिवर्तन आवृत्ती पाई गई।इस अवलोकन का मुख्य विन्दु स्प्लाइसोसोमल मशीनरी है जो कि स्प्लाइसोसोमल रेगुलेशन मे मुख्य भुमिका निभाती है। तथा M.D.S.रोग के रक्त केंसर के रूप में परिवर्तनके लिए उत्तरदायी होती है।

उत्परिवर्तन एवं रिकॉम्विनेशन के कारण उत्पन्न हुई जीनोमिक अस्थिरता रक्त केंसर का कारणहोता है। हाल ही में द्वितीयक स्प्लाइसींगभीजीनोमिक अस्थिरता के लिए जिम्मेदार सिद्ध हुई है। साधारणतः द्वितीयक स्प्लाइसींग युकेरियोटिक कोशिकाओं में मुख्य प्रकिया है जो की mRNA के विभिन्न आइसोफॉर्म बनाती हैतथा मनुष्य के विकास एवं विविधता की प्रकिया को प्रभावित करती है। इस प्रकिया का अवरोधन कई गंभीर बीमारियाँजैसे की रक्त केंसर को जन्म दे सकता है। युकेरियोटिक जीन जेनेटिक सुचनाओ को स्थानांतरित करने के लिए सबसे पहले Pre-mRNA के रूप में परिवर्तीत होते है जो कि इण्ट्रान्स (सुचना रहित भाग) एवं एक्सॉन (सुचना सहित भाग) से मिलकर बने होते है। इसके इण्ट्रान्स भाग को हटाने का काम स्प्लाइसोसोमल जीन्स करते है जो कि संख्या मे ज्यादा होते है और मिलकरएक मशीन की तरह काम करते है। इस बहुआण्विक मशीन में स्प्लाइसोसोमल जीन्स के अलावा पॉच सुक्ष्म राइबोन्युक्लीयों प्रोटीन् 1, U2, U4, U5 और U6 (SnRNAS) भी शामील होते है। कायीक कोशिकाओं में स्प्लाइसोसोमल मशीनरी के जीन्स मे उत्परिवर्तन जैसे इण्ट्रान रिटेन्सन्एक्सॉन स्कीपींग्एक्सॉन इलोंगेशन तथा ट्रंकेटेड प्रोटीन मे क्रीप्टीक साइट सक्रीयन आदि के कारण कई प्रकार के परिवर्तीत स्प्लाइस ट्रान्सक्रीप्ट वन सकते हैजो की रक्त केंसर तक पैदा कर सकते है। चुँकि M.D.S. एक विषमरूपीम रोग् है और अध्ययनों के अनुसार गलत स्प्लाइसींग से उत्पन्न हुई कोशिकीय विषमता रोगों का कारण हो सकती है। इसलिए M.D.S. में स्प्लाइसोसोमल उत्परिवर्तन को कैन्द्र मानकर इसके विस्तृत प्रभाव एवं संभावीत चिकीत्साउपचारपर अध्ययन किया जा रहा है।

Pre-mRNA स्प्लाइसींग की जटिल प्रक्रिया- पोस्ट ट्रान्स क्रिप्सनल प्रोसेसिंग में प्राथमिक ट्रान्सक्रीप्ट से परिपक्व mRNA वनने में इण्ट्रोन्स कोहटाकार स्प्लाइसोसोमल मशीनरी द्वारा एक्सान्स को आपस में जोडा जाता है। इस प्रक्रिया को pre-mRNA स्प्लाइसींग कहा जाता है।इस प्रकिया में स्प्लाइसोसोमल मशीनरी के साथ पाच सुक्ष्म प्रोटीन्स (SnRNPs) भी भाग लेते है। तथा क्रम वद्ध रूप से जुड़ते और निकलते हैजिसके कारण RNA-RNA, RNA-protein और protein-protein का जुड़ाव होता है।

स्प्लाइसींग रेगुलेशन के लिए सीस एलीमेण्ट जो की pre-mRNA में होते है। तथा ट्रान्स एलीमेण्ट जो इनसे जुड़ते है कि मुख्य भुमिका होती है। इसके साथ ही स्प्लाइससाइट की सही पहचान हीजीन एक्सप्रेसन का मुख्य भाग होता है।युकेरियोट में मुख्य रूप से दो तरह के इण्ट्रोन्स होते है एक U2 टाईप और दुसरा U12 टाईप। इनमे से U2 टाइप ही सवसे अधिक मात्रा (99%) में पाया जाता है। स्प्लाइसींग मुख्यतः तीन चरणो E, A तथा B में संपन्न होती है।

स्प्लाइसींग का नियमन– पुर्व शोध आधारितज्ञान के अनुसार माना जाता है कि स्प्लाइसींग का नियमन इनहेन्सर और मन्दक के माध्यम से होता है। ये मात्र १0nt लम्वाई के टुकडे होते है जो कि या तो एक्सॉन में या फिर इण्ट्रॉन में विखरे या समुह के रूप में उपस्थित होते है। यह नियामक प्रोटिन से जुढकर स्प्लाइस साइट का उपयोग कम या ज्यादा कर स्प्लाइसींग का नियमन करते है। इन सिस एलीमेण्टस को चार समुहो में वॉटा गया है। ये चारो समुह विभिन्न नियामक प्रोटिनों के साथ जुड़करस्प्लाइसींग का नियमन करते है।

रक्त कैंसर में स्प्लाइसोसोमल जींस के उत्परिवर्तन– M.D.S रोग में उत्परिवर्तीत स्प्लाइसोसोमल जीन्स मुख्य रूप से स्प्लाइसींग काम्पलेक्स E और A के भाग होते है। हाल ही में हुए एक अध्ययन के अनुसार जो कि टयुमर ऊंतक और नियामक (Control) ऊतक के एक्सॉन सिक्वेसींग पर आधारित थामें सावित हुआ है कि उत्परिवर्तन की प्रतिशतता सवसे ज्यादा उन स्प्लाइसोसोमल जीन्स में है जो कि स्प्लाइसोसोमल काम्पलेक्स के भाग है। कुछ मुख्य जीन्स जो कि M.D.S में उच्च आवृत्ति में उत्परिवर्तीत पाए गए है वह निम्नानुसारहै-

SF3B1: एम . डी . एस . में यह जीन मुख्य रूप से सर्वाधिक उत्परिवर्तीत पाया गया है। और गुणसुत्र कमांक 2q33.1 स्थिती पर पाया जाता है। यह जीन स्प्लाइसींग फेक्टर 3b प्रोटिन कॉम्पलेक्स की सवयुनीट एकके लिए कोड करता है। जो कि स्प्लाइसींग के शुरूआती पद में काम आती है। यह उत्परिवर्तन एम . डी . एस के RARS समुह के 70% मरीजों में पाया गया है।

U2AF1: यह जीन गुणसुत्र कमांक 21 की q22 स्थिती पर पाया जाता है। तथा U2 एक्सलरी फेक्टर कॉम्पलेक्स के लिए कोड करता है। जो कि Pre-mRNA कि इण्ट्रान ब्रांच साइट पर U2 SnRNP नियुक्तकरने के लिए आवश्यक होता है। हाई रिस्क M.D.S. के मरिजों में इस जीन में उत्परिवर्तन की आवृत्ति 8.7% पाई गई है। तथाइस उत्परिवर्तनके मरिजों में रक्त केंसर की सम्भावना तथा वुरी प्रोग्नोसिस (Bad-prognonsis) भी पाई गई है। साथ ही 20q del या -20 के मरिजों में इसकी आवृत्ति ज्यादा पाई गई।

SRSF-2: यह जीन गुणसुत्र कमांक 17 की q25.2 स्थिती पर पाया जाता है और स्प्लाईस साइट सिलेक्शन में भुमिका निभाता है। पुर्व अध्ययन के अनुसार यह जीन रक्त निर्माण तंत्र की कार्य क्षमताके लिए उत्तरदायी होता है।इस जीन में उत्परिवर्तन के कारण इसकी क्षमताप्रभावित होती है जिसके कारण रक्त केंसर होने का खतरा बढ़ जाता है।

ZRSF-2: यह जीन गुणुसुत्र X की p 22.2 स्थिती पर पायाजाता है। तथा यह जीन सकिय 3' स्प्लाईस साइट की पहचान करने के लिए आवश्यक है। जो की स्प्लाइसोसोमल तंत्रकीपरस्परक्रिया मे महत्वपुर्ण भुमिका निभाता है। इस जीन मे कोई मुख्य उत्परिवर्तन हॉटस्पॉट न होकर संपुर्ण जीन में ही उत्परिवर्तन होता है। हालाकि M.D.S. रोग की वृद्वि में इसका प्रभाव अभी ज्ञात नही है। हाल ही में M.D.S. में हुए अध्ययनो से ज्ञात हुआ है कि ZRSF-2 को यदि नॉर्कडाऊन किया जाता है तो U12 टाइप इण्ट्रॉन रिटेन्सन होता है। जब की U-2 टाइप इण्ट्रॉन अप्रभावित रहता है। साथ ही मज्जॉ कोशिका की गति एवं वृद्वि कम हो जाती हैऔर मायलोइड इरीथ्रोइड श्रृखंला की कोशिकाओ का विभेदन गडबड हो जाता है।

वर्तमान में MDS की चिकित्सा हेतु उपलब्ध औषधीयाँ- वर्तमान में कई प्रकार की औषधीयॉ जैसे – काइनेंज इनहीबिटर डण्अमेथाइल ट्रान्सफेरेज इनहीबीटर मरीजों को दी जाती है।इसके साथही नवीन कारक जो की कोशिकाओं की उपापचय कीयाओ का अवरोधन कोशिका – मृत्यू अवरोधन रक्षा तंत्र परिवर्तन एवं मन्दन करते है का उपयोग भी प्रयोगो के तौर पर M.D.S. में किया जा रहा है जैसे एजासीटीडीन, लिनालिनोनामाइड (वेरीनो स्टेट), SGI-110, ARRY-614 आदि। हालाकी ह्युमन स्टेम सेल ट्रान्सप्लाण्टेशन (HSCT) इलाज का एक प्रभावी तरीका है परंतु इसके हानीकारक प्रभाव जीवनशैली में परिवर्तन आदि कमियों के कारण ज्यादातर मरीजों में यह सम्भव नही हो पा रहा है। हालाकि कॉर्डब्लड और हेपलो आइडेन्टीकल मरीजों से प्राप्त मज्जॉ कोशिकॉए वृद्ध मरीजों में प्रभावी होती है। साथ ही ग्राप्ट मेनीपुलेशन जैसे Post-HSCT एजासीटिडीन एवं इम्यूनोसप्रेसीव का उपयोग कर HSCT का प्रयोग बढ़ायाजा सकता है।

स्प्लाइसोसोमल मंदक एवं स्प्लाइसोसोमल उत्परिवर्तन- नई पिडी की केंसर औषधीयाँ जो की मुख्य रूप से कुछ विशिष्ट मालिक्यूलर टारगेट जिनकी केंसर की वृद्वि में महत्वपुर्ण भुमिका होती है को कैद्रिंत कर बनाए जाते है कैद्रिंत थेरेपी की श्रेणी मे आती है। वर्तमान में मुख्यतः दो औषधीयाँ जो कि प्राकृतिक रूप से बनाई जाती है। इनमे से एक FR-901464 तथा दुसरी प्लेडीनोसाइर्डभ्ये दोनो ही औषधीयाँ कोशिकाओं को G1 तथा G2/M अवस्था में रोककर स्प्लाइसोसोमल मशीनरी के कार्य को बाधीत करती है। इसी प्रकार स्प्लाइसोस्टेडीनअएक दुसरा औषधीय यौगिक है जो SF3B1 की कार्यप्रणाली को प्रभावीत कर स्प्लाइसोसोमल मशीनरी के कार्य को बाधीत करता है।

उपसंहार एवं भविष्यमें उपचार की संभावनाए- स्प्लाइसोसोमलमशीनरी में उत्पन्न हुए नवीन उत्परिवर्तनो के जैविक प्रभाव एवं इनको लक्षितकरने वाली नई थेरेपी के सम्बध मे कई प्रश्न है जिनके उत्तर अभीज्ञात नही है। परंतु भविष्य में होने वाले अध्ययन जो कि इन उत्परिवर्तनों के कारण उत्पन्न होने वाले फीनोटाइप (आकारिकीपरिवर्तन) तथा जीन एक्सप्रेसन मे होने वाले परिवर्तन के संबध में ज्ञान बढाने में सहायक सिद्व होगे। तथा इस ज्ञान के माध्यम से स्प्लाइसोसोमलउत्परिवर्तन से होने वाले कैंसररूपांतरण के संबध में सुचना तथा इनका नवीन मालिक्युतर मेकेनिजम आधारित औषधीय उपचार सम्भव होगा। हालाकी जहा तक हमें M.D.S.की काम्पलेक्स क्लोनल हेटेरोजेनाइटी प्रकृति के संबध में सम्पुर्ण सुचना नही मिल जाती है तब तक केवल स्टेम सेल थेरेपी में संशोधन के माध्यम से ही M.D.S. का उपचार प्रभावी तरीके से किया जा सकता है।

LIBRARY & DOCUMENTATION SERVICES

LIBRARY BOOKS AND PERIODICALS

In 2018-19. Library subscribed 27 International and 10 National Journals both Print & Online on Hematology, Immunology, Genetics, Blood Transfusion, Hematogenetics, and Haemostasis. Library also subscribed to Online Databases and E-journal consortium like NML-ERMED, JGATEPLUS, ICMR e-journals and Web of Science database.

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Audio-visual services like video conferencing, webcasting/ webinars on SKYPE and ZOOM software are available for Scientific meetings, workshops, conferences, and seminars. A High tech seminar hall with a sitting capacity of 70 people with latest sound system and Wifi facilities are available for conducting various scientific and social programs.

LIBRARY UTILIZATION PARAMETERS

The library is installed with the latest automation software SLIM21 with advanced modules of Cataloguing, Acquisition, Circulation and OPAC system. Library barcode identity cards are issued to users for management of library resources. Library records are stored in library automation software for easy circulation of books and journals.

Retroconversion of library resources was ongoing using Infocuz digital library software a universal storage solution for archiving and mirroring in CD's/DVD's. Metadata like e-journals, seminar reports, thesis, and archival photographs were uploaded in this software. The digital data will be stored centrally in a server which can be searched throughout the LAN/WAN network. This software will keep abreast the Institute repository digitally.

Library services provided to the Scientists, Staff, and Students

1. Updated Library link at NIIH website: www.niih.org.in
2. Library Newsletter for 2018 was published in IIH Bulletin.
3. Resource sharing amongst nearby Medical and Research Libraries.
4. Optical fibre WI-FI Facilities for library and Institute.
5. Reprographic services on multi-digital color photocopier are provided to staff, students and visitors.
6. J-Gate Plus consortia for information interchange and retrieval among ICMR libraries.
7. Access to online full-text articles through NML-ERMED e-journal consortium, National Medical Library, New Delhi. i.e. www.ermed.in

8. Access to E-journals consortium of ICMR.
9. Access to the Web of Science database.

The recent addition of the books/journals to the library

1. Books - 23
2. Journals
   a) International - 27
   b) National - 10
3. E-journals - 20
4. Annual Reports - 16
5. CD-ROM/DVD - 12

Dr. D.S. Gangwar, Addl Secretary & financial advisor, Ministry of Health & Family Welfareduring his visit to the institute on 14th May, 2019.

Ms. Manisha Patwardhan, Technical Officer, was given farewell party at NIIH seminar hall on 30th April, 2019.

International Yoga Day celebrated at ICMR-NIIH Mumbai on 21st June 2019.

Training on HIV Testing and quality control at ICMR-NIIH from 6-9th Aug 2019.
Sixty four participants attended the same.

ICMR Task Force Training Program on Thalassemia and G6PD conducted at NIIH from 2-5th July 2019.
Nine participants attended the same.

ICMR Task Force Training Program on Thalassemia and G6PD conducted from 2-5th July 2019

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