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REVIEW

RNA processing and ribosome biogenesis in bone marrow failure disorders

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Bone marrow failure disorders (BMFDs), which are characterized by an early pro-apoptotic phase which results in faulty hematopoiesis and anemia, more often than not progress to outright acute myelogenous leukemia (AML). Recent findings have indicated that most if not all of these disorders have a very significant RNA processing component to their pathology. This review aims to highlight some of normal processes of RNA metabolism that have been recently demonstrated to be altered in BMFDs.

Keywords: RNA editing/splicing; hematopoiesis; leukemia; transcription; translation; apoptosis

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Introduction

Bone marrow failure disorders (BMFDs) are a heterogeneous and insidious group of hematological diseases whose symptoms inevitably include a mono- or pan-cytopenia with an elevated risk of developing acute myelogenous leukemia (AML), but these disorders may also involve other tissues outside the hematologic compartment. It is believed that bone marrow failure is the most characteristic symptom of these disorders, because it is the bone marrow and hematologic compartment the undergoes the most cell turnover and proliferation during the human lifespan making them more vulnerable [1]. The BMFDs are divided into two main groups, acquired and hereditary.

Acquired bone marrow failure disorders namely include myelodysplastic syndromes (MDS) and aplastic anemia (AP) where no known hereditary alteration is directly responsible for the development of the disorder [2]. These acquired disorders often arise later in life (>60 years of age; MDS) or following exposure to some form of cytotoxic stress, such as environmental toxins or chemotherapy. In contrast, hereditary bone marrow disorders involve the inherited mutation of a single gene or one of several related genes [1].

In all BMFDs, acquired and hereditary, there is a phase of intense selective pressure on the hematopoietic stem cell population resulting in enhanced apoptosis and inflammation (including the presence of cytotoxic cytokines) in the bone

marrow and mono- to pancytopenias in the periphery. In the case of MDS and FA, it has been clearly demonstrated that this phase is replaced over time by an anti-apoptotic phase where the hematopoietic progenitor cells lose their sensitivity to apoptotic stimuli [1-3]. This is often followed by the increased presence of immature hematopoietic progenitors in the periphery and the subsequent development of leukemia [2, 3].

The initial recognition of oncogenes/protooncogenes as causative agents in cancer and the identification of mutations, rearrangements, and altered expression of these same genes first focused major attention on the particular genes (ex. BCR-ABL in chronic myelogenous leukemia, CML). In reality, unlike CML, very few *de novo* cancers can be directly linked to alterations in known oncogenes. While some genetic alterations are repeatedly observed in certain cancers, the cancer of no two patients is the same. Later theories involved identification of cancer stem cells, or those cells that can give rise to tumors. Interestingly, cancer stem cells are more resistant to stress and therapy [4]. Recent data from bone marrow failure disorders, namely Fanconi anemia and MDS, have indicated that in hematological pre-malignancies that often give rise to AML, there is a process of natural selection taking place in the bone marrow [1-3]. Exposure to chronic stress and inflammation or hypersensitivity to inflammatory mediators (TNFα, IL-1α, IFNγ) promotes the growth suppression and apoptosis of hematopoietic progenitors in the bone marrow. Thus, HSCs and progenitors that survive and proliferate have, over time, acquired random gain of function mutations, leaving them with characteristics of cancer stem cells. This same process may explain the role of chronic stress and inflammation and the tight association of the innate immune system with cancer [2, 3].

While the selective pressures exerted in the bone marrow may favor random gain of function mutations, many of these alterations have a gross impact on RNA processing, splicing and ribosome biogenesis. This review aims to cover recent findings BMFDs that suggest altered RNA processing maybe at the heart of the selective (apoptotic) phase of BMFDs but may also facilitate the progression to AML.

Acquired bone marrow failure disorders

Acquired bone marrow failure disorders are those where no known hereditary alteration is directly responsible for the development of the disorder and typically occur later in life. As such, the initial cause of the disorder is not known but seems to heavily involve an immune component, either innate immune/inflammatory or autoimmune [5-7]. Acquired BMFDs namely include MDS AP.

Myelodysplastic syndromes (MDS)

Myelodysplastic syndromes are a heterogeneous group hematological malignancies that result in cytopenias in one or more of the hematologic lineages, with or without cytogenetic abnormalities. *De novo* MDS typically occurs in later life (>60 years of age) and is thus considered an age-related disorder. In contrast, MDS can also arise from the progression of other hematological malignancies including BMFDs. Moreover, therapy-related MDS (tMDS) is known to occur in individuals previously treated with chemotherapeutic agents for diverse types of cancer [7].

MDS is categorized by the International Prognostic Scoring System (IPSS) as low, intermediate-1, intermediate-2 and high based on the risk of the disease progressing to acute leukemia. In low-risk disease there is a propensity for hematologic stem cells and progenitors to undergo apoptosis with few blasts observed in the peripheral blood. This is gradually replaced during disease progression from low- to high-risk, where there is hypercellularity of the bone marrow and the appearance of blasts in the periphery [6, 7].

Diverse groups have demonstrated a role for stress/inflammatory signaling during progression of MDS to AML [2, 8, 9]. More recently, significant alterations in DNA methylation, chromatin modification, transcriptional regulation, DNA repair, signal transduction, sister chromatid cohesion and RNA splicing and ribosome biogenesis associated genes have been observed. Mutations in RNA splicing/processing genes are estimated to be present in 45-85% myelodysplasias [10]. Diverse groups have observed mutations in serine/arginine-rich splicing factor (SRSF)-2, splicing factor 3B subunit 1 (SF3B1), spicing factor U2AF 35 kDa subunit (U2AF35) and U2 small nuclear ribonucleoprotein auxiliary factor 35 kDa subunit related protein 2 (ZRSR2). Certain mutations are specific to particular subtypes of myelodysplasia [10-12].

Aplastic anemia

The cause of aplastic anemia is not well understood, but it is believed to result from a hyperactive immune response to the bone marrow [5]. As such the onset of aplastic anemia is rapid and devastating creating a pancytopenia. Without proper treatment, affected individuals usually succumb to the disease in a matter of months. Environmental exposure to pesticides and chemicals, as well as certain viral infections have been linked to the development of aplastic anemia, but is ultimately the unresolved immune attack of the bone marrow and hematopoietic progenitors involving cytotoxic T-lymphocytes and cytotoxic cytokines such as interferon

(IFN)-γ and tumor necrosis factor (TNF)-α that give rise to the disease symptomology [5, 13]. It should be noted that aplastic anemia, like MDS, can arise from a pre-existing/congenital condition such as a hereditary BMFD. Treatment usually consists of anti-inflammatory/steroids or bone marrow transplantation. Individuals who survive one bout of aplastic crisis are at an elevated risk (~50%) to experience another attack and/or, through clonal selection, to develop MDS and AML [5]. Thus, many of the alterations seen in surviving aplastic anemia patients tend to be those same alterations observed in MDS and AML (see above).

Hereditary bone marrow failure disorders

Hereditary bone marrow disorders which include: Fanconi anemia (FA), Diamond-Blackfan anemia (DBA), Shwachman-Diamond syndrome (SDS), Dyskeratosis congenital (DC), Kostmann syndrome (SCN) and Amegakaryocytic Thrombocytopenia (AT). All involve the presence of an inherited mutation in one particular gene (SDS, AT) or one of a set of genes involved in a common cellular process (FA, DBA, DC, SCN), which is ultimately responsible for the observed disease phenotype [1]. In those BMFDs where a mutation in more than one gene can causes the same disease, the pattern of inheritance and the penetrance of the disease phenotype may vary greatly.

While the hereditary BMFDs and individuals that have survived a bout of aplastic anemia show increased risk for the development of MDS, individuals with any bone marrow failure disorder demonstrate an elevated risk for the development of AML [1, 5]. Interestingly, it is this characteristic of progressing from a pro-apoptotic state to a pro-proliferative one that has modified the cancer stem cell theory and begun to revolutionize the way in which cancer as a disease is perceived to develop, by introducing the principle of clonality [3, 6]. Clonality is the offspring of natural selection occurring in the bone marrow in response to the chronic presence of innate immune/stress/inflammatory signaling.

Fanconi anemia

Fanconi anemia is a rare, congenital, recessive disorder characterized by diverse overt abnormalities (microcephaly, microphthalmia, abnormal tumbs and radii, and slow growth) as well as unexplained cytopenias and early onset of myelodysplasia, aplastic anemia, or AML. Affected individuals may also present with head-and-neck, esophageal and gynecological cancers at an atypically young age [1, 14]. Fanconi anemia can arise from the mutation of any of approximately 21 known genes and cases are grouped based on the gene mutated. The majority of FA patients fall into groups A, C or G with respective mutation in the FANCA, FANCC or FANCG genes. Individually, the encoded FA proteins carry-out diverse functions in metabolism, oxidative stress, cellular signaling and transcriptional regulation to name a few, but together they either form the core complex required for double-strand DNA break repair or are essential for its regulation [14]. As such, FA patients are hypersensitive to DNA damaging agents, especially DNA cross-linking agents such as mitomycin C. In addition, bone marrow stem cells and early progenitors are hypersensitive to the effects TNFα and IFNγ, and demonstrate elevated innate immune/inflammatory signaling similar to chronic inflammation [2, 14, 15]. As these patients begin to progress to MDS and AML, there is a concurrent loss of sensitivity to the same cytotoxic stresses the bone marrow stem cells and hematopoietic progenitors were previously hypersensitive, again pointing to clonal evolution [2, 3, 9, 15, 16].

While unlike many of the other congenital BMFDs, genes mutated in FA have no known direct link to RNA splicing/processing (although DNA damage may favor alternate splicing of RNAs encoding proteins that aid in the damage response), their effects on DNA damage repair predispose the cell to accumulate mutations in other genes, including those involved in RNA processing. In addition, the constitutive activation of PKR which is known to interact with intermediates of RNA processing, including splicing factors, ribosomal proteins, RNA helicases and ribonucleoproteins, may select for survival alterations in these intermediates [17]. Thus, it may be inferred that the alteration in RNA processing and splicing genes in MDS represents a compensatory result of clonal selection which favors survival.

Diamond-Blackfan anemia (DBA)

Diamond-Blackfan Anemia (DBA) is a rare congenital bone marrow failure disorder often noted within the first year or two after birth. DBA is mainly characterized by a profound anemia, but like FA, other congenital defects (growth retardation, cranialfacial abnormalities, defects in the heart and urinary system) are observe in a significant portion of patients (up to 50%). These individuals have an increased incidence of developing MDS and AML as well as some other forms of cancer [1, 18]. DBA appears to result from altered ribosomal RNA (rRNA) processing which, in turn, affects ribosome biogenesis of the 40S and 60S ribosomal subunits and alters mRNA processing/transport and translation; thus having pleiotropic effects on cellular growth and survival [18].

To date, DBA has been associated with diverse mutations in the following ribosomal proteins: RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPS27, RPS29, RPL5, RPL11, RPL26, RPL27, RPL35A and deletion of RPL15; and other novel mutations have been reported in RPL3L, RPL6, RPL7L1T, RPL8, RPL13, RPL14, RPL18A and RPL31. Approximately 50% of DBA patients contain mutations in RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPS29, RPL5, RPL11, RPL26, RPL35A or GATA. Mutations in RPS19 alone account for ~25% of these patients [19]. The other 50% of cases have no yet identified mutation, but are almost assuredly mutations associated with pre-rRNA processing and ribosome assembly.

There is some evidence that altered ribosome biogenesis may induce an inflammatory component. In patients bearing a RPS19 deficiency, the levels of GATA1 were found to be reduced in the erythroid progenitor population while p53 and TNFα expression were increased in the non-erythroid progenitors [18, 20]. Inhibition of TNFα in a zebrafish model of RPS19-deficiency was able to rescue the observed anemia, suggesting TNFα expression has a significant role in the observed phenotype [20].

Shwachman-Diamond Syndrome (SDS)

Shwachman-Diamond syndrome is an autosomal recessive disorder which initially manifests as an exocrine pancreatic dysfunction but then results in bone marrow failure and skeletal abnormalities. Approximately 20% of SDS patients will progress to MDS while another 25% will develop AML [1]. In almost all cases (~90%), patients carry a mutation in the Shwachman-Blackfan-Diamond syndrome (SBDS) gene. This protein is required for the proper maturation and nuclear export of the 60S ribosomal subunit to which it is often found associated [1, 21]. In addition to the 60S ribosomal subunit, SBDS has also been found in association with 28S rRNA and nucleophosmin (NPM) [21]. The general consensus is that mutations in SBDS result in altered 60S ribosome biogenesis and an enhanced sensitivity to stress.

Dyskeratosis congenita (DC)

Dyskeratosis congenital (DC) is a highly rare multi-system progressive bone marrow failure disorder that can either present an autosomal dominant, autosomal recessive or X-linked inheritance pattern base or the causative mutation. Its penetrance can vary widely extending from barely detectible to severe as in the case of Hoyeraal Hreidarson syndrome [1, 22]. Dyskeratosis presents with a triad of symptoms which include, reticulated skin hyperpigmentation, nail dystrophy and mucosal leukoplakia and results in premature death resulting from bone marrow failure, respiratory dysfunction or malignancy.

While dyskeratin (*DKC1*), which encodes for a protein involved both in the small nucleolar ribonucleoprotein (H/ACA snoRNP) and in the telomere complexes, and whose mutation is responsible for the X-linked form of DC, is by far the better studied; other proteins involved in the H/ACA snoRNP complex (*NOP10* and *NHP2*), the telomerase ribonucleoprotein complex shelterin (*TINF2*); *TERT*, the telomerase reverse transcriptase; *TERC*, which encodes the RNA component of TERT and whose mutation is responsible for the autosomal dominant form of DC; *WRAP53*, which delivers TERC to the telomerase as well as binds Cajal body RNAs (scaRNAs) and regulates p53 mRNA levels post-transcriptionally; *RTEL1*, which is involved in telomere elongation; and *CTC1*, a subunit of the CTC complex which terminates TERT activity and recruits DNA polymerase for complement strand synthesis, are also implicated [1, 22]. All these proteins are involved in telomere maintenance, while several are also directly involved in small ribonuclear RNA processing, resulting in pseudouridylation of the snRNAs. It is still not clear as to whether alterations in the telomerase complex itself is the underlying cause of DC or whether telomere shortening in DC patients also has a major component linked to processing of RNAs required for the telomerase complex [23, 24]. As might be expected tissues with the greatest proliferative/turn-over rates are the most affected by DC.

RNA processing

RNA processing refers to many aspects of RNA metabolism including, splicing (alternative splicing), post-transcriptional nucleotide modifications (methylation, pseudouridylation, deamination), folding, stability, degradation, cellular localization and transport. A large number of cellular proteins and RNAs are dedicated to these processes, and alteration of any of these may result in disease. The following sections aim to examine the role of RNA processing defects in bone marrow failure disorders by examining the role of the diverse proteins altered in these hematopoietic disorders in RNA processing.

mRNA splicing

In eukaryotic cells, the splicing of exons to remove the introns is mandatory for the proper synthesis of mature mRNA transcripts and their encoded proteins [25, 26]. In the nucleus the pre-mRNA or primary transcript enters its first post-transcriptional modification through the association of the spliceosome complex. Splicing requires both *cis*- and *trans*-acting factors. The *cis*-acting factors consist of RNA

sequences 5' and 3' of the intron-exon junction and the branch-point region located near the 3' splice site. In addition to these, sequences present in the introns and exons serve as splicing enhancers or silencers. The trans-acting factors consist of the small nuclear ribonucleoproteins snRNPs U1, U2, U4/6 and U5 as well as close to 300 additional proteins [25, 26].

Splicing initiates with the ATP-dependent binding of U1 snRNP to the 5' splice site of the intron. This interaction is stabilized by members of the serine/arginine-rich (SR) protein family. At the same time, the SF1/BBP protein and the U2 auxiliary factor (U2AF) associate with the branch point region at the 3'-end. The 35-kDa subunit of the U2AF heterodimer binds to the 3' splice site, while the 65-kDa subunit of U2AF serves to bridge the SF1/BBP and 35-kDa subunit of U2AF. This initial recognition of the 5' and 3' splice sites forms what is referred to as the E-complex [26]. At this point the U2 snRNP associates in an ATP-dependent manner with the break point region forming the A-complex and is stabilized by the association of SF3 (A and B) proteins as well as U2AF. SF1/BBP is displaced and replaced by other SF3 proteins. A pre-assembled U4/U6/U5 complex is then recruited to the A-complex to form the B-complex. Structural alteration of the spliceosome results in the destabilization of the U1 and U4 interactions and these snRNPs are lost resulting in an active spliceosome complex or B'-complex which initiates the first catalytic step of splicing to form the C-complex [26]. The second catalytic step in splicing occurs through structural modification of the spliceosome RNP component, which may associate or disassociate from complex. The final phase results in the release of U2, U5 and U6 snRNPs and the release of the spliced mRNA and intron [26]. This process sequentially occurs to form the mature mRNA, but in some cases not all introns are removed or not every exon is included to form the final mRNA. This is often a result of alternative splicing.

Alternative splicing of mRNA is a fundamental process to enhance the possibility of gene expression and increase protein diversity, where numerous interacting components of the spliceosome complex and associated hetronuclear RNPs (hnRNPs) are involved with *cis*-acting elements in the primary transcript. Any impairment of this mechanism is associated with failure of normal cellular function with a final outcome of disease [27]. It is becoming more evident that for a majority of genes one single primary transcript strand does encode only a single protein but rather diverse isoforms of the same protein, each having specific functions in given tissues. Not only can alternative splicing affect the amino acid sequence of the resulting protein, affecting its function/activity or localization, but it can also influence the translation efficiency and stability of the encoding mRNA and thus regulate protein expression post-transcriptionally but pre-translationally. This mechanism is finely regulated at developmental stages in different tissues, and an alteration in regulation of alternative splicing is now linked with several human diseases, including leukemia and pre-leukemic states such as MDS [28]. Recent findings of frequent mutations of gene involved in RNA splicing in myelodysplasia has, therefore, identified a significant event that highlights how cancer develops by targeting critical cellular functions during the process of clonal selection [29]. It also gives an interesting view into the mechanism of cancer specific alternative splicing [30]. Evidence exists implicating splicing factor mutations as founder mutations in MDS and having a role in MDS progression to AML [31-33]. SF3B1, U2AF1, SRSF2 and ZRSR2 are splicing factors that carry recurrent somatic mutations in MDS and are components of the E/A splicing complex that coordinates 3' splice site recognition during the early phase of pre-mRNA processing [10, 34, 35]. Alternative splicing may be the point of departure for the discovery of novel diagnostic and prognostic biomarkers and more than that for new therapeutic strategies of diseases, primarily cancer.

Serine/Arginine-rich splicing factors (SRSFs)

The serine/arginine (SR) splicing factor family is comprised of 12 phylogenetically conserved and structurally related proteins involved in the control of constitutive and alternative pre-mRNA splicing, as well as in other aspects of gene expression [36]. SRSFs contain one or two RNA-recognition motif at the N-terminus and one SR domain, which serves as an intermediary in the interaction with other proteins, at the C-terminus [36]. SRSF1 and SRSF2, which have been extensively investigated, serve pivotal roles in cell cycle regulation, genome stability, translation and pre-mRNA splicing [37].

Activity of the SR proteins is highly regulated by extensive and reversible phosphorylation of serine residues carried-out by CDC-like kinase (CLKs), SRSF protein kinase (SRPKs), and pre-mRNA splicing 4 kinase (PRP4K). These phosphorylations modulate protein–protein interactions within the spliceosome and regulate the activity and sub-cellular distribution of SR proteins. Therefore, changes in the phosphorylation state of SR proteins during growth factor/cytokine stimulation or stress (inflammation, cytotoxic cytokines) play a critical role in the control of their activity [36, 38].

Somatic mutations involving core components of the RNA splicing machinery have been detected in myelodysplastic syndrome (MDS). SRSF2, one of the SR proteins composed of a RNA recognition motif (RRM) domain and a RS

domain, frequently binds to splicing enhancer sequences (SES) and acts as a general splicing activator. In addition, SRSF2 is involved in transcriptional activation, RNA stability, mRNA transport, and mRNA translation [39]. Moreover, SRSF2 also has a role in the regulation of DNA stability; in fact, loss of SC35 in mouse embryonic fibroblasts induces G2/M cell cycle arrest and genomic instability, consequent at least in part from p53 hyperphosphorylation and hyperacetylation [40]. Heterozygous mutations in SRSF2 are common in patients with myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML) and are associated with adverse prognosis. The most frequent mutation that occurs in SRSF2 affects its binding affinity and induces abnormal splicing of primary hematopoietic regulators [41-43].

Thol *et al*., reported that in an analysis of 193 MDS patients, mutations are present in at least 1 of the investigated genes involved in the splicing machinery (i.e., SRSF2, U2AF1, ZRSR2, and SF3B1) in 34.7% of all investigated patients, suggesting mutations in genes that encode proteins involved in the splicing pathway are common in MDS. Mutations in SF3B1 and SRSF2 were the most prevalent aberrations identified in MDS patients; SRSF2 mutations were also associated with a negative prognostic impact [44]. SRSF2, U2AF1, and ZRSR2 mutations were present in all subtypes of MDS and were not associated with a specific IPSS risk profile or cytogenetic aberration. In this study, the authors also presented evidence that mutations in SRSF2 are related with mutations in RUNX1, a member of the core binding factor family of transcription factors and one of the most frequently mutated genes in a variety of hematological malignancies, as well as IDH1, the gene coding for isocitrate dehydrogenase, an enzyme of the citric acid cycle; thus stabilizing a link with metabolism and the mitochondria [44].

Splicing factor 3B1 (SF3B1)

SF3B complex is a constituent of the essential U2 snRNP splicing factor [26]. The SF3B1 protein is one of seven subunits (SF3B1-5, SF3B14 and PHF5A) of the SF3B complex [45]. Unlike SRSFs, SF3B1 mutations are associated with specific subtypes of MDS. Mutations in SF3B1 abrogate the role of different genes related to mitochondrial metabolism and they are found in a high percentage (70-90%) of MDS patients whose disease is characterized by the presence of ring sideroblasts, including both refractory anemia with ring sideroblasts (RARS) and refractory cytopenia with multilineage dysplasia and ring sideroblasts. Thus, patients harboring SF3B1 mutations present an increased number of ringed sideroblasts due to impaired synthesis of heme [11, 46].

SF3B1 mutations are generally more prevalent in low-risk MDS and have been shown to be independent predictors of favorable clinical outcome in MDS [46]. It has been shown that SF3B1 knockdown in four myeloid cell lines resulted in inhibition of cell growth and disruption of the cell cycle [47]. Recently, Paolella *et al*. found that wild-type SF3B1 is a non-driver CYCLOPS (Copy-number alterations Yielding Cancer Liabilities Owing to Partial losS); in other words, partial loss of SF3B1 pre-disposed cells harboring this loss to cell death upon exposure to stimuli that further reduce SF3B1 expression, thus partial loss of this gene results in a pro-apoptotic state. The CYCLOPS dependency is distinct from SF3B1 dependencies targeted by current spliceosome inhibitors [48]. SF3B1 mutations can be seen in 80% of patients with MDS-RS-SLD (MDS-RS with single lineage dysplasia) and 40% of patients with MDS-RS-MLD (MDS-RS with multilineage dysplasia), with the percentage of BM RS often correlating directly with the SF3B1 mutant allele burden. The survival of patients with MDS-RS-MLD is inferior to that of MDS-RS-SLD, but better than that of patients with MDS-MLD without BM RS or SF3B1 mutations [45]. Therefore, SF3B1 initially may represent a compensatory protective mutation; a middle of the road alteration that while enhancing the pro-apoptotic state, hampers progression to AML.

U2 auxiliary factor (U2AF)

The U2AF heterodimer plays a vital role in defining functional 3′ splice sites in pre-mRNA. Mutations are present in the U2AF complex, particularly in the U2AF1 gene (which encodes for the U2AF35 subunit) in blood disorders and other human cancers [49]. The U2AF complex consists of 65kD and 35kD subunits, and, as a heterodimer, is one of the best-characterized splicing factors in higher eukaryotic cells. Multiple mutations in both U2AF65 and U2AF35 have been reported to associate with blood disorders, particularly MDS [7, 44]. Several studies using unbiased genomic analysis of isogenic human and murine cells expressing ectopic mutant or wild-type U2AF1 have revealed that mutations in U2AF1 consistently alter the 3′ splice site (SS).

U2AF1 mutations are associated with abnormal splicing of genes involved in functionally important pathways, including cell cycle progression and RNA processing, and with mutations in epigenetic regulators ASXL1 and DNMT3A [44]. These mutations are most frequent in more proliferative phenotypes, including MDS/MPN and high-risk MDS [50]. U2AF1 (U2AF35) together with SRSF2 mutations are associated with refractory cytopenia with multi-lineage dysplasia (RCMD), refractory anemia with excess blasts (RAEB), and to high-risk leukemic transformation. Moreover, U2AF1 and SRSF2 present mutations associated with shorter survival [51].

Ilagan *et al*., has revealed that downstream targets of U2AF1 mutations may promote pathogenesis through

Table 1. Heterogeneous Ribonuclear Proteins (hnRNP) and Their Functions

hnRNP Protein	Function
hnRNPA/B	Consists of A0, A1, A2/B1 and A3. They are important for mRNA translation, splicing and localization of A2RE/RTS element containing transcripts. Also directs miRNAs to exomes for cellular export (requires sumoylation). A3 binds single-stranded telomeric DNA.
hnRNP C	Members of this family can oligomerize, due to a leucine zipper in the auxiliary domain. This feature allows hnRNP C members to bind unstructured RNAs and sort them by size. Also stimulates MYC translation.
hnRNP D	Also known as AUF1, this family is composed of four proteins that bind AU repeats and stimulate mRNA decay. In contrast, binding of these protein complexes to the TERT mRNA stabilizes it.
hnRNP E	Also referred to a poly C binding proteins (PCBP). Consists of E1 (PCBP1) and E2 (PCBP2), which bind 3' UTRs to stabilize the mRNA. Also act in alternative splicing and stimulate the transcription of some genes. In addition, these protein complexes stimulate IRES-mediated translation of some transcripts. E1 and E2 control $p21^{Waf1}$ mRNA half-life. The related poly C binding proteins E3 and E4 do not fall into the classification of a hnRNP due to their constitutive cytoplasmic localization, but E3 has been demonstrated to be a transcriptional repressor.
hnRNP F	This protein which is similar to the hnRNP F protein works in combination with other hnRNPs to promote alternative splicing.
hnRNP G	Also referred to as RBMX, this family consists of sex-linked proteins. HnRNP G is located on the X chromosome and is ubiquitously expressed. A related member RBMY is a hnRNP G family member located on the Y chromosome and expressed only in testis. These proteins regulate splicing allowing for the inclusion of some exons or exclusion of others in diverse transcripts. One target is exon 10 splicing of *tau* transcript.
hnRNP H	This protein which is similar to the hnRNP F protein works in combination with other hnRNPs to promote alternative splicing.
hnRNP I	Also known as polypyrimadine tract binding protein (PTBP)-1, hnRNP I interacts with polypyrimidine stretches at the branch point region 5' to exons. It is involved in RNA stability, translation and pre-RNA processing (regulating splicing). HnRNP I also control alternate 5'-3' splice site usage. In addition, hnRNP I can sequester miRNAs or block access to their complementary site on the mRNA.
hnRNP K	Similar to hnRNPs E1 and E2, hnRNP K has multiple nuclear-cytoplasmic functions including: splicing, transcription, and silencing of mRNA. HnRNP K interacts with GSK3β mRNA during osteoclast differentiation.
hnRNP L	Similar to hnRNP I, hnRNP L is involved in many aspects of RNA metabolism including mRNA stabilization and translation. HnRNP L is also involved in inducible exon skipping.
hnRNP M	HnRNP M acts as part of the spliceosome complex and is known to regulate splicing of fibroblast growth factor (FGF)-2 mRNA as well as others. Similar to hnRNP Q.
hnRNP P2	Also known as RNA binding protein fused in sarcoma/translated in liposarcoma (FUS/TLS), this hnRNP is best known for its association with the neurodegenerative disease amyotrophic lateral sclerosis (ALS).
hnRNP Q	Also known as SYNCRIP, hnRNP Q is associated with mRNA maturation. Three isoforms exist (Q1, Q2 and Q3) all produced by alternative splicing of the same transcript. Similar to hnRNP M.
hnRNP R	HnRNP R is known to enhance transcription of the *fos* oncogene; it is also known to regulate CAP-independent translation by binding to the IRES element of target mRNAs.
hnRNP U	Closely related to hnRNP R, hnRNP U is mainly involved in transcription and alternative splicing.

Information for this table was retrieved from UniProt/SwissProt database and Geuens,T. *et al*. (2016). *Human Genetics* 135: 851-867.

quantitative alterations in splicing related to several cellular pathways, enlightening the normal function of U2AF1's zinc finger domains. They show that specific alterations in 3' splice site recognition are crucial factors that promote the molecular pathology of MDS and related hematological disorders [52]. Park *et al*., show that the U2AF35 mutant promoted the usage of a distal poly(A) site in Atg7 mRNA, thus generating a longer, inefficiently translated transcript. Decreased ATG7 led to mitochondrial dysfunction including genomic instability, which induces cells to acquire secondary mutations disposing them to transformation [53].

Heterogeneous ribonuclear proteins (hnRNPs)

Heterogeneous ribonuclear proteins assist in the maturation of pre-mRNA primary transcripts to mature mRNAs during nuclear to cytoplasmic transport, and their subsequent translation, by influencing alternative splicing, mRNA stability, transport and folding [54]. There are 20 major types of hnRNPs and several minor families that most likely serve as regulators of the major hnRNPs. The hnRNPs contain a nuclear localization sequence (NLS) and are thus, primarily localized to the nucleus; but this localization is highly dependent on post-translational modifications which result in cytoplasmic localization of the hnRNPs. In addition to the NLS, the hnRNPs contain at least one or a combination of four types of RNA-binding domains (RBDs): RNA recognition motif (RRM), RRM-like, glycine rich with an RGG box, or KH-domain. The specificity of RNA-protein binding within hnRNPs is entirely dependent on the three dimensional structure of the protein surrounding the RBD, with diversity dictated by the combination of different RNA-binding motifs present [54]. Many of the hnRNPs are known to be part of the spliceosome complex and influence splicing of particular mRNAs or their alternative splicing [54]. Binding of some hnRNPs to the 3'-end untranslated region (UTR) is known to stabilize some mRNAs while targeting the degradation of others. Moreover, some hnRNPs sequester specific mRNAs or suppress their translation [54]. In addition to the mRNAs, the hnRNPs also have major effects on miRNAs. Other than these processes, the hnRNPs influence translation of specific mRNAs as well as the process of internal ribosome entry site (IRES) dependent translation [54]. Table 1 lists some of the hnRNP types and their known roles. While the hnRNPs have not been directly linked to BMFDs or cancer, they are major regulators of alternative splicing and their expression is known to influence the transcription and translation of multiple oncogenes. In addition, hnRNP E

members regulate tumor suppressor $p21^{Waf}$ and p63 mRNA stability as well as the epithelial-mesenchymal transition (EMT). On the other hand, mutation of other hnRNPs, such as hnRNP P2 (FUS), are associated with neurodegeneration [54].

Adenosine deaminases acting on double-stranded RNA (ADARs)

RNA editing is an important post-transcriptional mechanism occurring in a wide range of organisms, which alters the primary RNA sequences through the insertion/deletion or modification of specific nucleotides. In humans, the most common type of RNA editing is mediated by the ADAR enzymes converting adenosine into inosine, which is interpreted by the cellular machinery as guanosine, within dsRNA coding and noncoding regions of the primary transcript. A-to-I RNA editing within mRNAs and ncRNAs (long ncRNA and miRs) contributes to increases in the human RNA/protein landscape [55]. A-to-I RNA editing events within pre-mRNAs can generate or destroy splice sites and alter codons thus increasing proteome diversity. Other than modifying mRNAs at splice sites and coding regions, modification at the 5' and 3' untranslated regions (UTRs) can alter translation and stability of the mRNA, respectively. In addition, modifications of miRNAs or other ncRNAs can alter their target specificity or function. Recently, it has been reported that the A-to-I RNA editing frequency is massively increased from mouse to human [56]. Fascinatingly, in humans, most A-to-I RNA editing events (≥90%) occur within *Alu* inverted repeats, located preferentially in gene-rich regions.

In mammals, there are three highly conserved members of ADARs, ADAR (also known as ADAR1 or DSRAD), ADARB1 (ADAR2) and ADRB2 (ADAR3). The ADAR enzymes share similar domain structures: dsRNA-binding domains (RBDs), three in ADAR1 and two for ADAR2 and ADAR3 and a catalytic deaminase domain at the C terminus [57]. A-to-I RNA editing provides a powerful mechanism for fine-tuning the gene transcripts playing an essential role during development as shown by $Adar1^{-/-}$ and $Adar2^{-/-}$ knockout mice, which are embryonic or post-natally lethal, respectively [55, 57].

The ADAR proteins work as homo- and heterodimers and the altered expression/activity of these RNA modifiers likely contributes to the alterations in splicing observed in hematologic malignancies. Specifically, ADAR1 plays an essential role in embryonic development, especially within the hematopoietic lineages [58]. Post-natal deletion of ADAR1 in normal hematopoietic cells selectively depleted hematopoietic progenitor cells compared with more primitive cells [59]. Moreover, it has been shown that ADAR1-deficient hematopoietic stem cells (HSCs) were unable to reconstitute irradiated recipients or form differentiated cell colonies [59]. Indeed, ADAR1 in hematopoietic stem cells controls both type I and II interferon-inducible transcripts and prevents apoptosis [60]. More recently, it has been shown that ADAR1 editing activity is essential also for normal erythropoiesis as Adar1-deficient erythroid cells display a profound activation of innate immune signaling and high levels of cell death [61]. Given the biological relevance of RNA editing in mammals it has been postulated that its deregulation could be linked to a variety of human disorders. In particular, RNA editing has been documented in several human cancers even though only recently a real connection between a RNA editing and cancer has been shown. One of the first cancers to be associated with altered A-to-I RNA editing was acute myeloid leukemia (AML) [62]. AML is a fast growing form of leukemia affecting the myeloid line of blood cells. The authors reported that in low-differentiated CD34(+)/CD117(+) blasts isolated from acute myeloid leukemia patients, the PTPN6 transcript was abnormally edited at multiple sites leading to an aberrant PTPN6 transcripts with an altered SH2 domain which inhibited the PTPN6 protein from acting as tumor suppressor gene [62]. It has been demonstrated that ADAR1 is also involved in chronic myeloid leukemia. Indeed, Wang and collaborators reported that ADAR1 is required for Bcr-Abl leukemia cell survival *in vivo* and that silencing ADAR1 results in a rapid leukemic cell loss [63]. These studies elegantly uncover the role of ADAR1 in myeloid leukemia cells and indicate ADAR1 as a promising molecular target for CML-directed therapeutic interventions [63]. While ADAR1 seems to play a major role in disease affecting hematological compartment recently a role for the ADAR2 enzyme has also been recently demonstrated. Chronic lymphocytic leukemia (CLL), is characterized by B cells growing in an uncontrolled manner with patients showing a down-regulation of important tumor suppressor microRNAs miR-15a and miR-16-1, miRNAs that are controlled by ADAR2 [64].

RNA helicases

Many of the processes in which the above mentioned proteins are involved require the unwinding of RNA-RNA duplexes or RNA-DNA heteroduplexes. This requires the activity of RNA helicases, most of which are classified as either DEAD-box (DDX) or DEAH-box (DHX) helicases based on a conserved sequence in the helicase domain [65]. In addition, other RNA helicases possessing variations of these sequences or more primative RNA helicases are also present in humans. In general these enzymes unwind the RNA secondary structure to aid in diverse processes: splicing, translation initiation, pre-mRNA processing, transcription,

Table 2. A Selection of RNA Helicases and Their Function

RNA Helicase	Function
DDX1	DDX1 is an RNA helicase acting on RNA-RNA and RNA-DNA duplexes. It may be involved in DNA double-strand break repair of actively transcribed regions of DNA. Act as a co-activator of NF-κB dependent transcription by associating with RELA. May also be involved in 3'-end cleavage and polyadenylation during pre-mRNA processing. This gene is often found amplified in cancer with that of MYCN.
DDX2	Also known as eIF4A-I or DDX2A, DDX2 interacts with other translation initiation factors to favor translation initiation by binding as a complex to the 5'-end of the mRNA and assisting ribosome binding. Often overexpressed in diverse cancers including melanoma and hepatocellular carcinoma.
DDX5	Also known as RNA helicase p68, DDX5 is involved alternative regulation of pre-mRNA splicing. It participates in altered splicing of *c-H-ras*. Its helicase activity is required for the inclusion of *tau* exon 10 and it acts as a transcriptional co-activator for the estrogen receptor (ESR1) and the androgen receptor (AR). It synergizes with DDX17 and SRA1 RNA to activate MYOD1 transcriptional activity during skeletal muscle differentiation. It also acts as a transcriptional co-activator for p53 and is involved in the p53 DNA damage response and p53 dependent apoptosis. Moreover, it also acts as a transcriptional co-activator for RUNX2 and is involved in osteoblast differentiation. Can be involved in promotor specific transcriptional repression through a mechanism likely involving HDAC proteins.
DDX6	Also known as p54/RCK, DDX6 regulates the translation of mRNA by decapping specific mRNAs, resulting in the degradation of these transcripts.
DHX9	Also known as RNA helicase A, DDX9 acts on dsDNA and dsRNA to alter the secondary structure allowing for protein-nucleic acid interaction. In complex with CBP and RNA pol II it initiates transcription in certain genes. DDX9 is also a component of the CRD-mediated complex that stabilizes *c-myc* transcripts. Along with DDX5, DDX9 inhibits 3'transcriptional termination of circadian target genes. DDX9 positively regulates HIV LTR-mediated transcription and gene expression, interferes with BRCA1 function when overexpressed, and up-regulates p16 expression. DDX9 is often overexpressed in lung carcinoma.
DDX10	DDX10 has a possible role in ribosome biogenesis. It is involved in the Inv 11(p15q22) observed in therapy related AML and MDS. This inversion results in a DDX10-NUP98 fusion protein.
DDX17	Exist as p72 or p82 isoforms. Transcriptional co-activator for the estrogen receptor ESR1. Act in synergy with DDX5 to stimulate MYOD1 transcriptional activity and is likely involved in skeletal muscle differentiation. Associates with U1 snRNP and promotes alternative splicing.
DHX32	Plays a role in pre-mRNA splicing and is often down-regulated in ALL.
DDX43	Also known as HAGE. Its role is unknown. Up-regulated in diverse cancers.
DDX47	DDX47 associates with pre-rRNA precursors and has a role in their processing and ribosome biogenesis. Likely has a role in mRNA splicing and stability. Associates with the E1E4 protein of HPV type 16. Is highly involved in apoptosis.
DDX48	DDX48 which is highly similar to DDX2A is also known as DDX2B or eIF4A-II. It is a core component of the splicing-dependent exon junction complex (EJC). Involved in translational enhancement of spliced genes by stabilizing spliced mRNA at the exon-exon junctions. Modifies the splicing of BCL2L1/BCL-X inhibiting the formation of BCL-X_s. Found as a common member of the nuclear matrix. It is involved in cranial-facial development and is often found up-regulated in gastric cancer.
DDX53	Also known as CAGE. Its function is unknown.

Information for this table was retrieved from UniProt/SwissProt database and Abdelhaleem,M. (2004). *Biochimica et Biophysica Acta* 1704: 37-46.

ribosome biogenesis and RNA-protein nuclear-cytoplasmic transport. These proteins are more often than not overexpressed in diverse cancers. Others such as DDX10 and DHX32 have been shown to be involved in chromosomal alterations in leukemia or down-regulated in ALL, respectively [65]. Table 2 lists some of the known RNA helicases and their roles.

rRNA processing and ribosome biogenesis

Ribosome biogenesis is one the most costly processes of the cell in terms of energy, and the cell goes to great lengths to maintain strict control over the process. The final product results in a functional ribosome, which in eukaryotes consists of 40S and 60S subunits contains 4 species of processed ribosomal RNA (18S, 28S, 5.8S and 5S) and 79 ribosomal proteins (RPs) [66-68]. The mature 40S subunit consists of 18S rRNA and 33 RPs, while the 60S subunit consists of 28S, 5.8S and 5S rRNAs and 43 RPs. In addition to these, over 200 other non-ribosomal proteins and 75 snoRNAs are required for ribosome synthesis.

Ribosome biogenesis is a highly dynamic process in which, transcription of the pre-rRNAs, processing/modification of the pre-rRNAs, association of RPs to the pre-rRNAs, proper folding of the pre-rRNAs and transport of the maturing ribosomal subunit to the cytoplasm are all combined [66, 67]. The process initiates with the synthesis of the rRNAs. While the 5S rRNA is transcribed by RNA pol III from multiple genes into the nucleoplasm and then migrates to the nucleolus, the 18S, 5.8S and 28S rRNAs are transcribed by RNA pol I as a single precursor RNA from tandem repeats of the gene into the nucleolus. The initial pre-rRNA transcript maintains a secondary structure at the newly synthesized 5'-end which acts as a platform for the binding and association of an initial set of RPs. As this portion of the pre-rRNA contains what will become 18S rRNA of the 40S subunit, the RPs associating with the 5'-end are small RPs or RPSs. The initial binding of the RPSs and the processing at the external transcribed spacer (ETS) as well as modifications of the rRNA by ribose methylases (C/D box snoRNPs) and polyuridylases (H/ACA box snoRNPs) energetically favors an rRNA structure that forms a platform for the next round of RPs to associate [66, 67]. At the same time, processing of the 5'-end of the 18S rRNA is being conducted, processing and cleavage at the 3'-end of the 18S rRNA is occurring within the internal transcribed

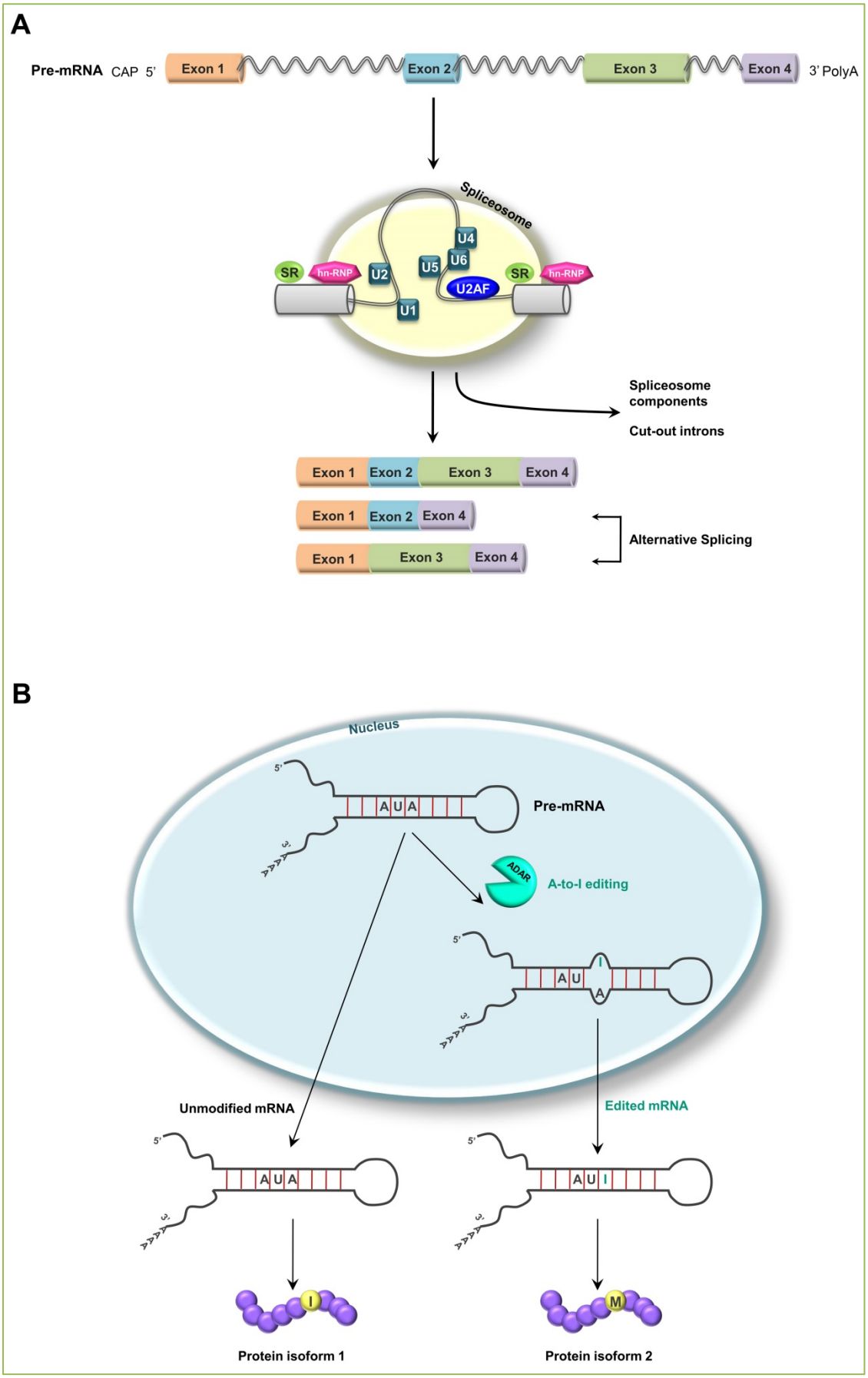
A
Pre-mRNA
CAP 5'
Exon 1
Exon 2
Exon 3
Exon 4
3' PolyA
Spliceosome
U4
U6
U5
U2
U1
U2AF
SR
hn-RNP
SR
hn-RNP
Spliceosome components
Cut-out introns
Exon 1 Exon 2 Exon 3 Exon 4
Exon 1 Exon 2 Exon 4
Exon 1 Exon 3 Exon 4
Alternative Splicing
B
Nucleus
5'
A U A
3'
Pre-mRNA
ADAR
A-to-I editing
A U I A
Unmodified mRNA
Edited mRNA
A U A
A U I
I
M
Protein isoform 1
Protein isoform 2

Figure 1. RNA splicing mechanisms increase the coding capacity of the genome. (A) Splicing and/or alternate splicing of the primary transcript lead to the production of diverse protein isoforms that may differ in tissue expression, cellular localization, physical properties and function. Alterations in expression or mutations in proteins involved in these processes have been found in most bone marrow failure disorders and contribute to the pathology of the disorder and the progression to AML. (B) RNA editing can induce amino acid changes that alter protein coding (as shown); alter the 5' UTR or 3' UTR of mRNAs changing their translation rates or stability; respectively; alter the specificity of miRNA, and thus change its target, or alter the miRNA recognition sites in targets allowing the target RNA to by-pass processing. More often than not RNA editing alters splice recognition sites and thus changes mRNA splicing site selection.

sequence (ITS1) to liberate the assembling 40S subunit from the 60S subunit that is now being assembled and beginning to be transported to the cytoplasm [66, 67]. Virtually the same process occurs for the formation of the maturing 5.8S and 28S with the ITS2 (between 5.8S and 28S sequences) initially beginning to be cleaved and processed before cleavage and processing of the 3' ETS of the pre-rRNA. At this point, the associating RPs are RPLs as they will become part of the 60S subunit. The process, marked by rounds of rRNA processing and folding and rounds of RP association, continues to occur until the last of the RPs for each subunit are finally incorporated in the cytoplasm. Due to the ability of RNA to assume diverse energy structures at equilibrium, diverse modes of assembly can be occurring simultaneously including the synthesis of kinetically dead-end products that are aborted and targeted for degradation and recycling by the TRAMP4/5 complex [67].

In addition, to the RPs incorporated into the ribosome and the rRNA modifying proteins, a number of proteins associate with the maturing ribosome in the nucleus to ensure that the ribosome does not prematurely assemble. Once exported into the cytoplasm these chaperons disassociate from the ribosomal subunits, allowing for ribosomal subunit assembly and the association of mRNA and translation initiation factors (IFs) [21, 66, 67, 69].

Alteration of ribosome biogenesis can have mild to dire consequences depending on the defect and its penetrance, and often affects tissues which have the greatest rates of proliferation and turn-over, including the skin and hematopoietic precursors in the bone marrow, especially erythroid precursors.

Ribosomal subunit proteins (RPs)

The ribosomal subunit proteins are divided into two groups based on the ribosomal subunit of which they are a part. Those associating with the 40S subunit are referred to as RPSs or small ribosomal subunit proteins, while those associating with the 60S subunit are referred to as RPLs or large ribosomal subunit proteins [66, 70]. Several lines of evidence indicate that the main purpose of the RPs is to stabilize rRNA folding and structure, thus assisting in processing: (i) the RPs are RNA-binding proteins which are dependent on RNA structure and conformation, (ii) there are very few protein-protein interactions between the RPs, (iii) while many of the RPs possess a tail which inserts into the ribosome core, the core is mostly hollow, with the exception of the rRNA species, and these protein tails do not interact with the mRNA substrate [66].

In contrast, the RPs may have other trans-regulatory functions. It has been demonstrated that certain RPs preferentially regulate the expression of NF-κB, p53, p21, Myc and some nuclear receptors. Others have been demonstrated to bind to the untranslated regions of mRNAs resulting in changes in mRNA stability or other transcript-specific outcomes. RPL40 is required for the efficient translation of many stress response proteins as well as vesticular stomatitis virus (VSV) mRNA, and RPL38 has been shown to affect the translation of some homeobox mRNAs [70].

The best known ribosomopathy is Diamond-Blackfan Anemia (DBA; *see above*). While some forms of DBA result from a complete loss of a RP, the majority of cases result from mutations that create a haploinsufficiency of the affected RP. Mutations in RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPS27, RPS29, RPL5, RPL11, RPL26, RPL27, RPL35A and deletion of RPL15 as well as other novel mutations in RPL3L, RPL6, RPL7L1T, RPL8, RPL13, RPL14, RPL18A and RPL31 have been reported. Approximately 50% of DBA patients contain mutations in either RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPS29, RPL5, RPL11, RPL26, RPL35A or GATA; RPS19 mutations alone make-up 25% of the cases of DBA [19]. In addition to DBA, 5q-syndrome in MDS presents a haploinsufficiency of RPS14 [71].

Two of the first RPs to associate with the pre-rRNA at the 5'-end are RPS7 and RPS24, which are required to initiate processing and cleavage of the pre-rRNA at the 5'-ETS; thus low levels of these proteins block or retard rRNA processing resulting in failed maturation of the 5'-end of the 18S rRNA. Similarly, depletion of the levels of RPS17 and RPS19, which are required for ITS1 cleavage, result in failed processing of 3'-end of the 18S rRNA. This failed ribosome

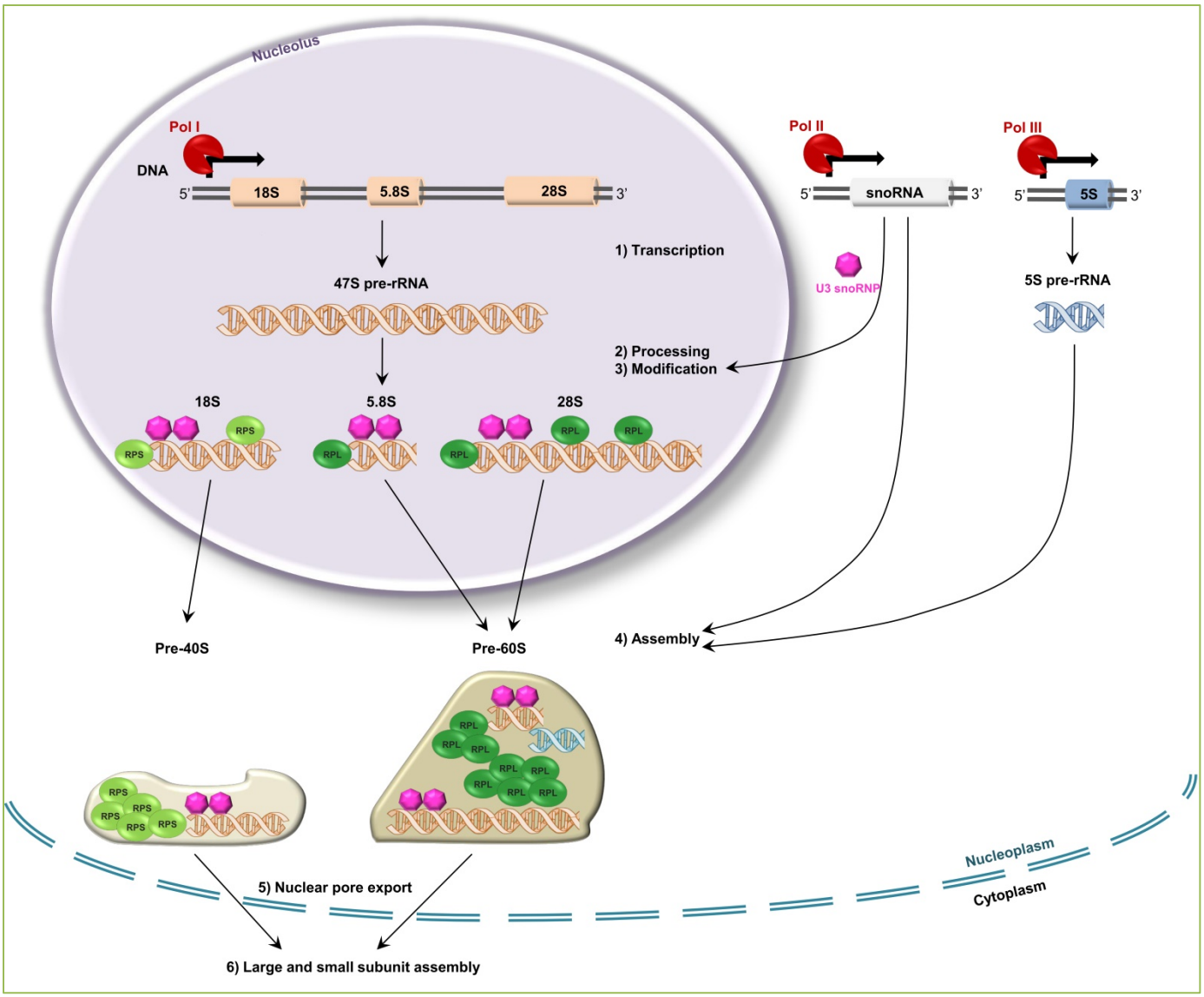

Figure 2. Ribosome biogenesis requires the simultaneous synthesis of rRNA, post-transcriptional modification of rRNAs, ribonucleoprotein assembly and nucleo-cytoplasmic transport. The diverse classes of proteins and ribonuclear protein complexes and where they intervene in the process of ribosome biogenesis are shown. The proteins involved facilitate the processing, modification and packaging of the rRNA species into the 40S and 60S subunits. Other proteins maintain these subunits separate until they have been transported to the cytoplasm where the combine to for the 80S ribosome.

biogenesis results in a pro-apoptotic state in affected cells apparently through the augmentation of p53 expression [70].

The expression of RPs as well as RNA pol I and III is under the translational control of mTOR and the eIF2α kinases (PKR, PERK, GCN2 and HRI) in response to growth factors, nutrient conditions and stress. Under growth conditions mTOR activity favors the enhanced expression of both RPs and the RNA polymerases resulting in sufficient expression of the components necessary for the ribosome biosynthesis. Under stress conditions, reduced mTOR activity and phosphorylation of eIF2α results in reduced translation and expression of the polymerases as well as the RPs [2, 70]. Interestingly, under the conditions of haploinsufficiencies of the RPs, the process of pre-rRNA maturation is altered resulting in an increasing pool of unassimilated RPs. These free RPs, including a complex consisting of 5S rRNA, RPL5 and RPL11, have been shown to associate with MDM2, the p53 ubiquitinase, and sequester it from p53, thus enhancing p53 protein stability [20, 70]. In addition to this mechanism, other mechanisms, including DNA double-strand (ds) breaks due to replication stress and AMPK activation, due to ATP depletion, have been proposed to lead to increased p53 expression. Thus, the enhanced level of p53 places the affected cells into a pro-apoptotic state where they are hypersensitive to certain stresses [70].

Other than enhanced p53 expression, other mechanisms may explain the pro-apoptotic and poor proliferative state observed following the loss or reduced expression of RPs. Loss of RPL5 or RPL11 was shown to lead to reduced cyclin expression, and therefore, reduced proliferation. Additionally, cells deficient in RPS19 and RPL11 were demonstrated to be predisposed to oxidative stress [70]. In addition, an innate immune component has been observed. RP loss/reduction enhances the expression of innate immune genes including interferon and TNFα, which is known to contribute to the hematopoietic failure in RPS19 deficiency, likely through IRES-mediated translation [20]. Moreover, RP depletion affects the insulin signaling pathway [70]. In fact, certain DBA patients present with symptoms similar to insulin resistance observed in pre-diabetic patients. Such a finding may relate to the constitutive activation of PKR, a stress-activated/innate immune eIF2α kinase that is key in phosphorylating the insulin response substrate (IRS1) resulting in insulin resistance [72, 73]. Interestingly, PKR is also required for the pro-apoptotic effects of interferon and TNFα [2, 15].

Shwachman-Bodian-Diamond syndrome protein (SBDS)

About 90% of patients presenting with Shwachman-Diamond anemia have a mutation in the SBDS protein. The gene encoding SBDS is located on chromosome 7q11 and is immediately adjacent to its pseudogene, SBDSP, which is 97% identical to SBDS but contains deletions and nucleotide changes that prevent the expression of a functional protein. Interestingly, it is a recombination with this pseudogene that results in SBDS mutations in 75% of patients [21]. The SBDS gene encodes a 250 amino acid (29 kDa) protein that contains no known structural domains. The exact function of SBDS is unclear, but it apparently has a role in both ribosome biogenesis and genomic stability. SBDS is expressed ubiquitously in tissues and is localized throughout the cell with a particular preference for the nucleolus where ribosome biogenesis occurs. Mouse knock-out models and the fact that a common early truncation mutation at nucleotide 183 (TA>CT) is observed only in individuals heterozygous for the defect, suggest that loss of SBDS is embryonic lethal [21].

As is the case for most bone marrow failure disorders, the number of CD34+ hematopoietic cells in the bone marrow is reduced, and these cells show a reduced proliferative and colony forming capacity when compared to normal CD34+ hematopoietic cells. In addition, elevated p53 expression and increased apoptosis are observed in the bone marrow of SDS patients [21].

SBDS associates with Nip7, a 60S ribosomal subunit assembly factor and eIF6 associated with the 60S ribosome. In the assembling 60S ribosomal subunit, eIF6 serves in the proper maturation of the subunit and to inhibit the premature assembly of the 40S and 60S subunits to form the 80S ribosome. Mutational rescue data suggest that SBDS serves to disassociate eIF6 from the 60S subunit once it reaches the cytoplasm. This occurs by the SDBS-dependent recruitment of the cytoplasmic GTPase EFL1 to the eIF6-60S subunit complex. EFL1 promotes the dissociation of eIF6 from the 60S subunit allowing for 80S assembly [21, 69]. Thus, during ribosome biogenesis reduced expression of SBDS hampers the dissociation of eIF6 which has a much larger influence in cells that are more translationally active.

In addition to its role in ribosome biogenesis, SBDS has also been shown to associate with microtubules during mitosis. SBS patients often show increased incidence of mitotic abnormalities, with multi-polar spindles and centrosomal amplifications. Data from human fibroblasts in which SBDS was knocked-down recapitulated these mitotic findings, but only after two weeks; thus, it is unclear if these observations in patients are a direct result of SBDS reduction or a result of other changes downstream caused by prolonged reduction of SBDS [21].

H/ACA snoRNP complex

The H/ACA complex is involved in: the maintenance of telomeres, the pseudouridylation of rRNAs, ribosome biogenesis, splicing of small nuclear RNAs, and direct microRNA processing [74, 75]. The complex consists of a protein component which is directed to its target by the RNA component. The protein component consists of four proteins: dyskerin (DKC1), NOP10, NHP2 and GAR. Dyskerin is a pseudouridylase containing a catalytic domain and a distal PUA domain. NOP10 is an elongated protein that binds and stabilizes DKC1; while NHP2 is a RNA binding protein which recognizes and binds RNAs with a K-turn or K-loop stem-loop structure. Finally, GAR, a small basic protein, provides stereo-spacial regulation to the active complex [76, 77]. The small nucleolar RNAs (snoRNAs) associate with the PUA domain in DKC1 and the K-binding domain of NHP2. The sequences complementary to the target to be modified, present in the loops, flank the catalytic pocket of DKC1. In some cases, two stem-loop structures support the pseudouridylation of multiple targeted uridines in proximity to one another. The binding of the H/ACA snoRNP protein complex to the snoRNA unwinds and opens the guide RNA-target RNA complex to favor the accessibility of the target uridine to the DKC1 catalytic site, where DKC catalyzes the formation of a C5 glycoside isomer of uridine [75, 76, 78].

Unlike the hnRNPs whose RNA targeting is dependent on the proteins present in the complex and their respective RBDs, the H/ACA snoRNP complex has a finite protein component and thus the diversity of its targets is dictated by the associated guide RNAs [75, 76, 78]. These guide RNAs (snoRNAs or scaRNAs) all contain closely related H-box and ACA-box sequence elements and are composed of one or more stem-loop structures which are interrupted by an internal bulge with sequences complementary to those sequences flanking the site of pseudouridylation in the target (the exception is telomere RNA which is a snoRNA but does not direct pseudouridylation) [76, 78]. The pseudouridylation of rRNAs, targeted by the H/ACA RNP complex, occurs in regions critical for ribosome activity such as the decoding center or peptidyl transferase domain. Pseudouridylation affects translation fidelity, stop codon usage and is required for IRES-mediated translation [76, 78, 79]. Alterations in the H/ACA snoRNP complex is most prominent in leukemias but is also altered in some solid tumors, including: prostate, head and neck, and non-small cell lung cancer [79].

The snoRNAs are derived from intron sequences spliced-out of primary transcripts encoding "host proteins". Hypermethylation of the DNA encoding these introns can result in decrease expression of specific H/ACA snoRNAs. In addition, mutation of these sequences can affect target binding of the H/ACA RNP complex. These H/ACA snoRNAs are highly mobile elements and a translocation along with a mutation that changes the target specificity of the guide sequences can result in an increase in the target repertoire, thus the number of targets is infinite [76, 78]. The H/ACA snoRNAs are also involved in chromatin structures and the iRNA machinery.

While the expression of H/ACA snoRNAs is typically reduced in disease, mutations in the H/ACA proteins (DKC1, NOP10 and NHP2) have been associated with the rare bone marrow failure syndrome, dyskeratosis congenita (DC) [80]. The H/ACA snoRNP protein components are also required for the correct processing and trafficking of TERC, the RNA component of the telomerase reverse transcriptase holoenzyme (TERT). Interestingly, in a zebrafish model, Pereboom, *et al*., demonstrate that NOP10 loss can give rise in the cytopenia phenotype in DC through the destabilization of the 40S ribosomal subunit as a result of 18S rRNA defects resulting in the cooperation of RPS7 with MDM2 and the stabilization of p53 in HSCs [24]. This hematopoietic failure could be rescued by the loss of functional p53. These data revealed that the effect of deficiency of H/ACA RNP complex genes on hematopoiesis is mediated by p53. It has always been debated as to whether DC is a disease related to ribosome biogenesis or loss of telomere maintenance. While much of the phenotype likely does result from lack of telomere maintenance, the data presented by Zhang *et al*. and Pereboom *et al*., demonstrated that the associated anemia and hematopoietic failure results primarily from impaired rRNA processing and ribosome biogenesis and the subsequent stabilization of p53, common features also seen in DBA and SDS [23, 24].

Conclusions

The bone marrow failure disorders as a whole have shed significant light on general mechanisms involved in clonal selection and progression to cancer. Other than direct DNA mutations or epigenetic modifications, many of the alterations observed that occur in these diseases and their progression to AML involve intermediates of RNA processing and ribosome biogenesis, which influence protein expression and isoform selection, as well as genome stability. The result is a pro-apoptotic atmosphere with the enhanced possibility for further survival mutations, resulting in clones resistant to the safeguards of normal cellular growth and differentiation. While many of these processes are coming to light, more in-depth analyses of these processes, their role and how they are regulated, is needed in order to define therapeutic targets for disease intervention. Such knowledge will require a major input from basic scientific research.

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Conflicting interests

The authors have declared that no conflict of interests exist.

Author contributions

WB wrote manuscript, MP conceived figures, AG wrote manuscript, AB conceived figures, EF conceived figures, IF wrote manuscript

Abbreviations

BMFDs: Bone marrow failure disorders; AML: acute myelogenous leukemia; MDS: myelodysplastic syndromes; AP: aplastic anemia; CML: chronic myelogenous leukemia; HSCs: Hematopoietic stem cells; SRSF: serine/arginine-rich splicing factor; SF3B1: splicing factor 3B subunit 1; U2AF35: spicing factor U2AF 35; DC: Dyskeratosis

congenital; AT: Amegakaryocytic Thrombocytopenia; hnRNPs: Heterogeneous ribonuclear proteins; ADARs: Adenosine Deaminases Acting on Double-Stranded RNA.

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