U2AF1 in various neoplastic diseases and relevant targeted therapies for malignant cancers with complex mutations (Review)

QING NIAN[1*], YIHUI LI[2*], JINGWEI LI[1], LIYUN ZHAO[3], FERNANDO RODRIGUES LIMA[4], JINHAO ZENG[5], RONGXING LIU[6] and ZHIJUN YE[7]

[1]Department of Transfusion, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072; [2]Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology and Visual Sciences Key Laboratory, Beijing 100730; [3]Department of Pharmacy, Personalized Drug Therapy Key Laboratory of Sichuan, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072, P.R. China; [4]Université Paris Cité, CNRS, Unité de Biologie Fonctionnelle et Adaptative, 75013 Paris, France; [5]Department of Gastroenterology, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan 610000; [6]Department of Pharmacy, The Second Affiliated Hospital, Army Medical University, Chongqing 400000; [7]Department of Clinical Nutrition, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072, P.R. China

Received October 3, 2023; Accepted November 3, 2023

DOI: 10.3892/or.2023.8664

Abstract. U2 small nuclear RNA auxiliary factor 1 (U2AF1) is a multifunctional protein that plays a crucial role in the regulation of RNA splicing during eukaryotic gene expression. U2AF1 belongs to the SR family of splicing factors and is involved in the removal of introns from mRNAs and exon-exon binding. Mutations in U2AF1 are frequently observed in myelodysplastic syndrome, primary myelofibrosis, chronic myelomonocytic leukaemia, hairy cell leukaemia and other solid tumours, particularly in lung, pancreatic, and ovarian carcinomas. Therefore, targeting U2AF1 for therapeutic interventions may be a viable strategy for treating malignant diseases. In the present review, the pathogenic mechanisms associated with U2AF1 in different malignant diseases were summarized, and the potential of related targeting agents was discussed. Additionally, the feasibility of natural product-based therapies directed against U2AF1 was explored.

Correspondence to: Dr Rongxing Liu, Department of Pharmacy, The Second Affiliated Hospital, Army Medical University, 183 Xinqiao Road, Chongqing 400000, P.R. China
E-mail: lrx1173942453@126.com

Professor Zhijun Ye, Department of Clinical Nutrition, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, 32# W. Sec 2, 1st Ring Road, Qingyang, Chengdu, Sichuan 610072, P.R. China
E-mail: drye028@outlook.com

*Contributed equally

Key words: spliceosomes, U2 small nuclear RNA auxiliary factor 1, malignant disease, targeted therapy

Contents

1. Introduction
2. U2AF1 mutations in malignant haematological diseases
3. U2AF1 mutation in different solid tumours
4. Therapies targeting U2AF1
5. Discussion and perspectives

1. Introduction

Before precursor mRNA (pre-mRNA) becomes a protein, it needs to be transformed into a mature mRNA by intron splicing and exon splicing. This splicing process can be achieved by major and secondary spliceosomes (1,2). The major spliceosome comprises five small nuclear ribonucleoprotein complexes (snRNPs) known as U1, U2, U4, U5 and U6. Of these, U2AF1 is the U2 small nuclear RNA auxiliary factor 1 protein coding various spliceosome genes. This spliceosome is composed of 240 amino acids with a molecular weight of 35 kDa and is located on chromosome 21q22.3 (3,4). The RNA binding region and C-terminal serine/arginine-rich (RS) domain are composed of the U2AF1 structure, which binds to the AG dinucleotide in 3' splicing sites (3' SS) at the boundary introns and exons. The 1 crystal structure of U2AF1 includes zinc knuckles (ZnK1, ZnK2) and a central U2AF homology motif (UHM). It is an integral part of both constitutive and enhancer-subordinate RNA joining by straightforwardly intervening in collaborations between the enormous subunit and the proteins bound to the enhancers (Fig. 1) (5-8).

Mutations in U2AF1 have been found to be associated with a wide range of diseases, most notably haematological malignancies such as myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML) as well as various solid

tumours (9-11). However, although U2AF1 mutations are commonly observed in the former haematological malignancies, these mutations appear with a low frequency in solid tumours (Fig. 2) (12-14). Additionally, mutations in other splicing-associated genes, including SF3B1, SRSF2 and ZRSR2, have been associated with haematological malignancies, albeit with distinct and mutually exclusive tendencies across highly conserved domains (15-19).

Given recent developments in sequencing technology, an understanding of the molecular pathogenesis of U2AF1-associated diseases has become increasingly important. Hence, the structure and pathogenesis of U2AF1 mutations in different diseases were outlined in the present review and potential therapeutic strategies were presented.

2. U2AF1 mutations in malignant haematological diseases

In MDS. MDS is a heterogeneous group of clonal haematopoietic stem cell (HSC) disorders characterized primarily by deficiencies in the myeloid, erythroid, and/or megakaryocytic lineages, mono- or multilineage dysplasia, clonal dominance of abnormal immature cells, and an increased risk of secondary AML. The French, American and British divisions of AML subtypes are categorized as M0 through M7 based on the type of cell the leukaemia develops from and how mature the cells are. The World Health Organization system classification is AML with certain genetic abnormalities. The phenomenon of AML emerging from MDS has prompted numerous researchers to suggest that the two disorders are related, with similar pathogenic mechanisms and treatments (20-24).

Heterozygous somatic mutations in splicing factor genes (SF3B1, SRSF2, U2AF1 and ZRSR2) occur in >50% of all patients with MDS (25-29), with U2AF1 mutations ranging from 7-11% and having the highest incidence in AML and the worst prognosis among the four mutations (30,31). Structural changes caused by mutations in U2AF1 occur in two hotspots-S34 and Q157-in the first and second zinc finger domains, as illustrated in Fig. 3. These mutations are capable of inducing gain-of-function changes, which modify the 3' SS preference in a sequence-specific manner, determined by the nucleotides surrounding the AG dinucleotide responsible for the 3' SS core consensus motif (10,15,32-34). This suggests that U2AF1 mutations directly affect RNA binding and consequently influence exon inclusion. It has also been observed that the effects of different mutations may vary on different exons; for example, the U2AF1 S34 mutation prefers C or A more frequently in the-3 position when analysed in AML patients, whereas the Q157 mutation, involving the first zinc finger, shows preferential recognition of G over A in the +1 position. By combining comprehensive data from RNA sequencing and U2AF1 mutations with 3' SS recognition ability, a clearer understanding of the mechanism by which U2AF1 mutations affect splicing in patients with MDS can be established (10,12,35,36).

Dysregulation of cell growth and immunity is a hallmark of MDS (37). In accordance with several research studies, aberrantly spliced genes identified in MDS with U2AF1 mutations converge on disrupted cellular differentiation processes, including RNA splicing, RNA localization/transport, RNA binding, protein translation processes, ribosomal pathways, and mitochondrial dysfunction (31,38-40). Subsequently, an analysis of upstream transcriptional regulators using the Ingenuity Pathway Analysis (IPA) tool suggested the significant enrichment of genes regulated through transcriptional regulators (RICTOR, E2F1, HNF4A, MYC, MYCN and RB1, the major controllers of cell growth/cell cycle) in the aberrantly spliced genes (38,41,42) (Table I). Additionally, these studies revealed that MDS with U2AF1 mutations has ten significant transcriptional pathways, with the sirtuin signalling pathway leading the rankings (38,43). Sirtuins, including sirtuin1, sirtuin2 and other isoforms, are a class of proteins that regulate diverse physiological processes and are considered to be partially involved in MDS and other haematological malignancies (44-46). Furthermore, mitochondrial dysfunction has generally been associated with MDS pathophysiology. In one study, mice with mitochondrial dysfunction exhibited characteristics of MDS, including macrocytic anaemia (47). Mitochondrial dysfunction can be induced by mitochondrial DNA mutations, electron respiratory chain leakages, increased oxidative stress, dysfunctional TCA cycle enzymes and abnormal tumour suppressive signalling pathways (48). Park *et al* (49) reported that cells expressing the U2AF1 S34F gene had defective autophagy along with mitochondrial dysfunction and increased production of reactive oxygen species, causing genomic instability and an elevated frequency of spontaneous mutations. These sirtuins in the mitochondria, which additionally function as histone deacetylases, are involved in metabolism, inflammation, genome stability and cell proliferation control. In addition, their role in ageing, cancer and survival has been demonstrated in some studies (50,51).

The NF-κB signalling pathway has a significant role in determining the quiescent or active state of HSCs, and aberrantly active NF-κB signalling may promote malignant HSPCs (52). Balka *et al* (53) identified that U2AF1 mutations resulted in aberrant splicing of IRAK4, a serine/threonine kinase involved in toll-like receptor downstream signalling, giving rise to a longer isoform named IRAK4-L, which preferentially assembles signal transducers (for example, MyD88 and IRAK kinases) into a myddosome, causing a higher degree of NF-κB activation. Moreover, IRAK4-L participated in innate immunity and was found to be associated with a poorer prognosis. Subsequent studies also revealed that mutations in SF3B1 and SRSF2 had a similar effect, leading to hyperactive NF-κB signals via aberrant splicing of MAP3K7 and CASP8, respectively (19,54-56) (Fig. 4). Overall, the aforementioned evidence suggested that mutations in various splicing factors alter different targets at the pre-mRNA splicing level but share a common downstream signalling node leading to heightened innate immune signalling.

The dysregulation of splicing factor genes is associated with the formation of R loops, a pathogenic aberrant RNA-DNA hybrid structure derived from nascent invasion. Crossley *et al* (57) showed that the expression of U2AF1 S34F mutations in human cancer cells led to R-loop accumulation. Furthermore, treatment of U2AF1 S34F-expressing cells with ATR inhibitors has been identified to induce DNA damage and cell death, which can be enhanced by spliceosome inhibitors (58,59). Chen *et al* (60) and Pellagatti *et al* (38) reported ERCC8 and FANCM mutations in MDS patients with U2AF1 mutations that may be involved in the suppression/regulation of R-loop formation and the DNA damage response. Chen *et al* (61) found that impaired transcription inhibits release at transcription start sites (TSSs), likely due

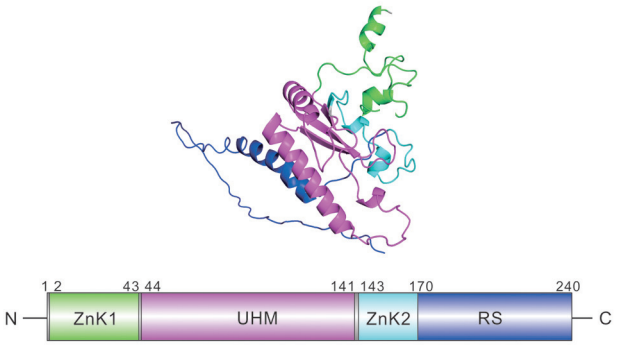

Figure 1. Protein structure of U2AF1. The crystal structure of the U2AF1 complex bound to 5'-UAGGU RNA is shown, with the N-terminal zinc finger in green (1-43 sites, labelled ZF1), the U2AF1 homology motif (44-141 sites, labelled UHM) in violet, the C-terminal zinc finger in azure (143-170 sites) and the RS domain in mazarine. U2AF1, U2 small nuclear RNA auxiliary factor 1.

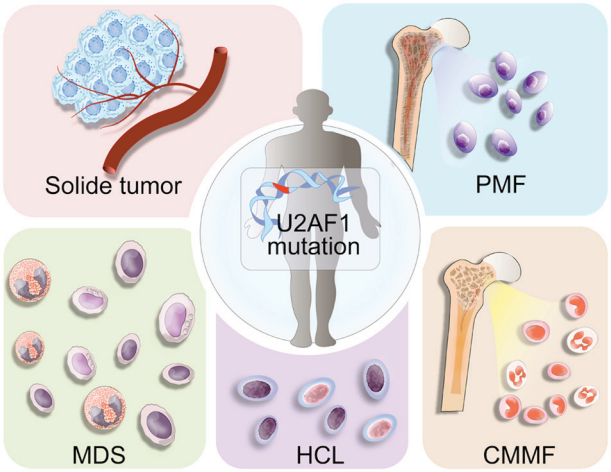

Figure 2. Mutations in U2 small nuclear RNA auxiliary factor 1 are observed in various types of haematological malignancies and solid tumours. MDS, myelodysplastic syndrome; PMF, primary myelofibrosis; CMML, chronic myelomonocytic leukaemia; HCL, hairy cell leukaemia.

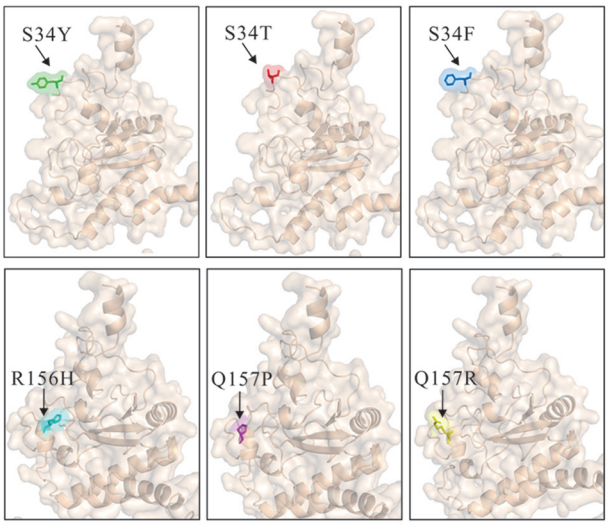

Figure 3. Structures of five variants of the U2 small nuclear RNA auxiliary factor 1 protein are presented in turn. S34Y, S34T, S34F, R156H and Q157P/R. The serine at position 34 is replaced by tyrosine, threonine and phenylalanine; arginine at position 156 is mutated to histidine; and glutamine at position 157 is altered to proline or arginine.

to the inability to extract P-TEFb, a kinase that phosphorylates the RNA polymerase II (Pol II) C-terminal domain, from the inhibitory 7SK complex to transcriptionally engaged Pol II at TSSs in SRSF2-mutant MDS, resulting in enhanced R loops. Interestingly, Pellagatti *et al* (38) and Boultwood *et al* (62) identified aberrant splicing of several genes, including SETX and ATR, involved in the suppression/regulation of R-loop formation in bone marrow $CD34^+$ cells of MDS patients with splicing factor mutations. Collectively, these data raise the possibility that R-loop-induced DNA damage may lead to deleterious mutations that promote clonal advantage in MDS pathogenesis.

In primary myelofibrosis (PMF). PMF is classified as a chronic myeloproliferative neoplasm characterized by bone marrow tissue fibrosis, enlargement of the spleen, and the occurrence of anaemia associated with the presence of nucleated and teardrop-shaped red blood cells. In PMF, the osseous components of bone marrow undergo a process known as osteosclerosis. Furthermore, fibroblasts secrete collagen and reticulin proteins, collectively referred to as fibrosis. Both of these pathological processes disrupt the usual functioning of bone marrow, leading to reduced production of various blood cells, including erythrocytes (red cells), granulocytes and megakaryocytes; the latter cells are responsible for platelet production. Thus, the combined effects of osteosclerosis and fibrosis in PMF significantly impair the normal haematopoietic activities of bone marrow (63-65).

Most cases of PMF are associated with mutations in the JAK2, CALR or MPL genes (Table I) (66). According to sequencing results, >15-20% of patients with PMF suffer from U2AF1 mutations, of which the two major mutation sites are Q157 and S34. These findings contrast with MDS, in which the incidence of S34 and Q157 mutations is low. Interestingly, the age distribution of patients with U2AF1 mutations is also different in MDS and PMF, with MDS patients tending to be younger and PMF patients tending to be older (67). In terms of clinical features, U2AF1 mutations are associated with anaemia, thrombocytopenia and poor disease prognosis. In MDS, U2AF1S34 mutations are more specifically associated with thrombocytopenia, while anaemia is more specifically associated with Q157 mutations (Fig. 3). Both types of mutations in PMF may cause anaemia, and Q157 mutations are more strongly associated with thrombocytopenia (68-70).

In chronic myelomonocytic leukaemia (CMML). CMML is a haematopoietic disorder that involves clonal proliferation of cells, presenting with diverse myeloproliferative and myelodysplastic characteristics. This complexity contributes to the heterogeneous nature of CMML. The tyrosine kinase inhibitor imatinib remains the primary targeted drug for the treatment of CMML, thus rendering it inevitable that resistance to this inhibitor will decrease its efficacy (71,72).

An analysis of a large cohort of patients with CMML revealed that the occurrence rate of U2AF1 mutations is ~9%. Patnaik *et al* (73) reported that the primary sites of U2AF1 mutations are S34F, Q157P/R and R156H. Among patients

Table I. The different mutation of U2 small nuclear RNA auxiliary factor 1 in malignant diseases.

Malignant diseases	Mutation site	Relevant gene	(Refs.)
MDS	S34, Q157	RICTOR, E2F1, HNF4A, MYC, MYCN, RB1, ROS, ERCC8, FANCM	(10,12,15,31-60)
PMF	S34, Q157	JAK2, CALR, MPL	(65-71)
CMML	S34F, Q157P/R, R156H	ASXL1, TET2, CBL, RAS, EZH2, TP53, IDH1/2DNMT3A, RUNX1, UTX	(74-76)
HCL	S34F, R156H	CD29, CD20, CD22, CD25, TRAP, Anx-A1, BRAF	(78,79)
Lung cancer	S34F	SLC34A2-ROS1	(80)
Prostate cancer	S34F	ARV7, MAPK, EZH2	(82,83)
Borderline ovarian tumors	S34T	TP53	(84)

MDS, myelodysplastic syndrome; PMF, primary myelofibrosis; CMML, chronic myelomonocytic leukaemia; HCL, hairy cell leukaemia.

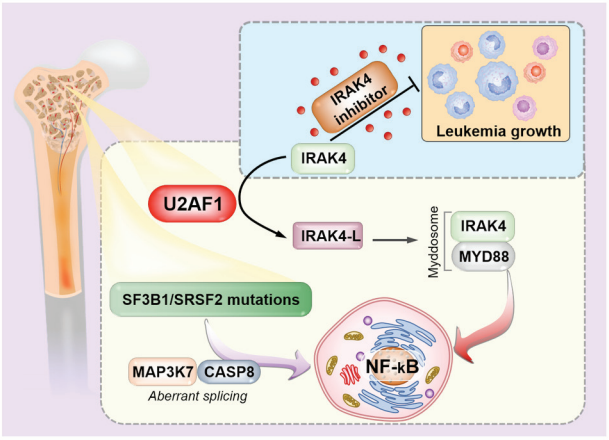

Figure 4. Link between U2AF1 and NF-κB signalling in MDS and AML. The isoform IRAK4 encodes the proteins IRAK4L and MyD88, which activate the NF-κB signalling pathway. In MDS/AML, a mutant U2AF1 splicing factor mediates the expression of IRAK4, and the inactivation of IRAK4 suppresses leukaemia cell proliferation. Moreover, mutations in the splicing genes SF3B1 and SRSF2 lead to aberrant regulation of MAP3K7 and CASP8, affecting NF-κB signalling expression. U2AF1, U2 small nuclear RNA auxiliary factor 1; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia.

with U2AF1 mutations, >50% carried the S34F mutation, while a subset of patients exhibited the Q157P and Q157R mutations, and only a minority had the R158H mutation (Fig. 3). Additionally, it has been demonstrated that other gene mutations, such as ASXL1, TET2, CBL, RAS, EZH2, TP53, IDH1/2, DNMT3A, RUNX1 and UTX, interfere with spliceosome genes and induce distinct amino acid missense mutations in U2AF1 (73-75). These insights suggest that targeting U2AF1 through drug design may hold promise for improving survival rates among patients with U2AF1 mutations.

In hairy cell leukaemia (HCL). HCL is a rare B-cell malignancy characterized by pancytopenia and splenomegaly, with an incidence of ~2% in adults. It is generally recognized as a subtype of chronic lymphocytic leukaemia and can be divided into two categories: Variant HCL (vHCL) and classical HCL (cHCL). vHCL is more prevalent, accounting for nearly 10% of HCL patients, whereas cHCL is rare among mature B-cell malignancies (76).

The presence of various markers, such as CD29, CD20, CD22, CD25, tartrate-resistant acid phosphatase (TRAP), annexin 1A (Anx-A1) and BRAF V600E mutations has been documented in the immune response of leukaemia cells and summarized in Table I. In the case of vHCL, several similarities with cHCL are notable, yet vHCL lacks expression of CD25, CD123, ANXA1, TRAP and BRAF V600E. Additionally, vHCL has been found to be more aggressive and less responsive to purine analogues.

vHCL exhibits several similarities to HCL. However, vHCL lacks the expression of CD25, CD123, ANXA1, TRAP and BRAF V600E. Furthermore, vHCL has been observed to be more aggressive and less purine analogue-sensitive (77). By detecting vHCL patients, Durham *et al* (78) found that the spliceosome U2AF1 was mutated, leading to its loss of function, and the major mutation sites were S34F and R156H, as demonstrated in Fig. 3. This is in contrast to cHCL, which only included BRAF V600E mutations. Thus, there is an important genetic difference between cHCL and vHCL. The aforementioned research provides evidence for potential novel therapeutic targets for vHCL, which may have implications for the future personalized treatment of the disease.

3. U2AF1 mutation in different solid tumours

In lung cancer (LC). As revealed in separate studies, there are recurrent U2AF1 mutations in LC, especially in lung adenocarcinoma (LUAD). U2AF1 mutations have been observed in ~3% of patients with LUAD, and the incidence of U2AF1 mutations in other LC subtypes appears to be lower, including squamous cell carcinoma and small cell carcinoma. In contrast to the aforementioned neoplastic disorders, the S34F mutation is the most pervasive hotspot in LC targeting the first zinc finger domain. Additionally, this mutation has been linked to altered splicing of genes such as GNAS, H2AFY, STRAP and PICALM in both LUAD and non-small cell LC patients. Based on an analysis of A549 cells, U2AF1 S34F mutations can lead to aberrantly spliced DNA damage repair factors (32,79).

In terms of pathogenesis, Esfahani *et al* (32) reported that U2AF1 S34F preferentially binds to and regulates splicing of introns containing CAG trinucleotides at their 3' splice junctions compared with its wild-type counterpart. Moreover, overexpression of U2AF1 S34F upregulates the expression of genes associated with epithelial-mesenchymal transition, thereby inducing cell invasion, which is mediated by preferential splicing of SLC34A2-ROS1 long isoforms (Table I) (32,80). These studies have demonstrated that in lung tumours harbouring U2AF1 S34F, a wild-type allele of the gene is invariably retained and is critically dependent on cell survival. However, a previous *in vitro* study observed that in cells harbouring multiple U2AF1 alleles, a mutation in one allele stimulates the emergence of the U2AF1 S34F mutation, leading to the development of U2AF1 'wild-type' cells. Consequently, these cells exhaust their wild-type alleles and give rise to isogenic U2AF1 mutant cells (80). This means that mutations in U2AF1 appear to be an important contributing factor in the formation of LC. Targeted therapies directed at U2AF1 mutations could represent a promising clinical option in the treatment of LC.

In other solid tumours. Prostate cancer is the second most commonly occurring cancer in men. A recent study identified U2AF1 mutations in prostate tumours, suggesting that this may be a putative biosignature for this disease (81). According to a recent study, downregulation of U2AF1 has been linked to poor prognosis and a correlation with androgen receptor variant 7 (ARV7). This downregulation can stimulate the proliferation of prostate cancer cells and lead to bicalutamide resistance by regulating intron splicing of ARV7. MAPK pathways have also been identified as having an administrative impact on U2AF1 and its regulation of ARV7 splicing (Table I). In summary, downregulation of U2AF1 can cause androgen resistance and increased proliferation of prostate cancer cells through the regulation of ARV7 splicing and an increase in MAPK1 expression, predicting a poor prognosis in patients with this cancer (82). A further study based on prostate tumour biopsies suggested, however, the overexpression of U2AF1 genes in association with EZH2 localized in mRNA and MAPK pathways (83).

Borderline ovarian tumours account for ~15-20% of all ovarian malignancies and arise from the tissue that encapsulates the ovary. However, in clinical practice, it is difficult to detect borderline mucinous tumours until advanced stages. In a case study of one patient with a borderline mucinous tumour, a novel mutation of U2AF1 was observed, with an alteration in amino acid serine in place of tyrosine, although such mutations are uncommon in ovarian tumours (occurring in <0.2% of cases) (84). On the other hand, TP53 mutations remain highly common in ovarian cancers. Moreover, U2AF1 and TP53 mutations can predict poor prognosis and a lack of remission. Consequently, inhibitors targeting U2AF1 are essential for these patients.

4. Therapies targeting U2AF1

Small-molecule modulator: NSC 194308. The small-molecule inhibitor NSC 194308 was synthesized by combining metallic Na and dry MeOH under an exothermic reaction with the evolution of hydrogen. Subsequently, (-)-cis-myrtanylamine was added and incubated for 16 h at room temperature before being reacted with isopropyl alcohol (iPrOH) and diethyl ether to form 2-chloro-N-(-)-cis-myrtanyl acetamidinium hydrochloride. The solution was stirred in ethyl (Et) alcohol and combined with Na2S2O3 in water R, and the compound's formation was monitored using thin layer chromatography (85). As a result, NSC 194308 was successfully synthesized and characterized as demonstrated in Fig. 5.

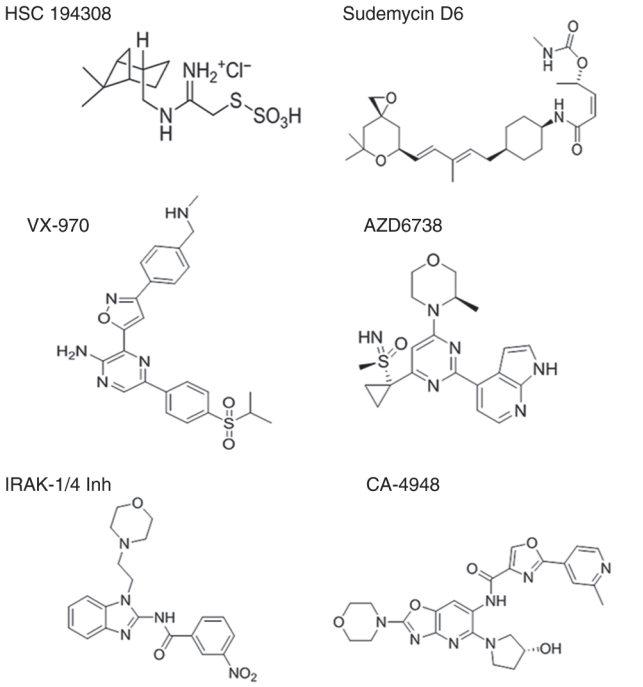

Figure 5. U2 small nuclear RNA auxiliary factor 1 targeting compounds in the present review.

The experiments of NSC 194308 in HeLa cells revealed that its addition directly reduced the splicing of the adenovirus major late promoter transcript substrate. An in-depth analysis of the intermediate stages of spliceosome assembly revealed that NSC 194308 repressed the pre-mRNA splicing process by inhibiting the gathering of spliceosomes at a U2AF-dependent checkpoint before tri-snRNP enlistment and catalytic activity. Subsequent chemical synthetic treatment with NSC194308 in K562 leukaemia cells, which had S34F mutations of U2AF1, was followed by high cell apoptosis (85). Collectively, these data suggested that NSC194308 is a promising agent for targeted therapy of leukaemia with U2AF1 S34F and other related mutations.

Synthesis of sudemycin D6. The Julia-Kocienski olefination is of paramount importance in regard to the synthesis of sudemycin D6. This form is derived from natural product spliceosome modulators (FR901464) through intricate chemical reactions, culminating in the formation of the (S,Z)-5-(((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)amino)-5-oxopent-3-en-2-yl-methylcarbamate structure of sudemycin D6. Notably, the biocatalytic synthesis of sudemycin D6 was first achieved using the Julia-Kocienski olefination methodology.

Table II. Targeting U2 small nuclear RNA auxiliary factor 1 drugs in clinical trials.

Drug	Target	Sponsor	Disease	Phase	Identifier
Immunotherapy	SF3B1, U2AF1 SRSF2	Sidney Kimmel, Comprehensive Cancer, Center at Johns Hopkins, Vanderbilt University, Bristol-Myers Squibb	Metastatic Solid Tumor	Interventional	NCT04447651
Emavusertib Azacitidine Venetoclax	SF3B1, U2AF1 SRSF2, ZRSR2	Curis, Inc.	Acute Myelogenous Leukaemia Myelodysplastic Syndrome	Phase 1 Phase 2	NCT04278768
Decitabine	SRSF2, U2AF1 ZRSR2	Samsung Medical Center	Myelodysplastic Syndrome	Interventional	NCT02060409

In this way, the Julia-Kocienski olefination is a pivotal step in the successful synthesis of sudemycin D6 (Fig. 5) (86).

Haematopoietic progenitor cells expressing CD34 were shown to be sensitive to sudemycin D6 treatment, resulting in altered pre-mRNA splicing and reduced survival of K562 cells with U2AF1 (S34F) mutants. The effects of this alteration on the cell cycle profile were examined *in vivo* using transplanted mice treated with sudemycin D6. The results demonstrated that expansion of U2AF1 mutant progenitors was attenuated, and a beneficial effect on the bone marrow of U2AF1-mutant mice was observed. Notable improvements were observed in biological pathways related to the immune response, leukocyte differentiation and cell proliferation regulation (87). The aforementioned study provided evidence that sudemycin D6 is a promising therapeutic strategy for targeting U2AF1 mutants and may have broader implications for the treatment of leukaemia.

Ataxia telangiectasia and Rad3-related protein inhibitor (ATRi). ATR is an essential protein-coding gene activated by DNA damage and DNA replication stress. The two known ATR inhibitors are VX-970 and AZD6738. VX-970, also known as berzosertib, has been used in clinical trials of patients with ovarian serous tumours, especially in U2AF1-mutant cancers. Its chemical name is 3-[3-[4-(methylaminomethyl) phenyl]-5-isoxazolyl]-5-(4-propan-2-ylsulfonylphenyl)-2-pyrazinamine. Furthermore, compound AZD 6738 (named ceralasertib) is a potent and effective sulfoximine morpholinopyrimidine ATR inhibitor. The detailed chemical structure of AZD 6738 is 4-(4-(1-((S(R))-S-methylsulfonimidoyl)cyclopropyl)-6-((3R)-3-methyl-4-morpholinyl)-2-pyrimidinyl)-1H-pyrrolo(2,3-b)pyridine (Fig. 5). Moreover, AZD6738 can inhibit ATR through the loss of downstream phosphorylation of CHK1 at Ser345 (88).

Adding ATRi to U2AF1S34F-expressing HeLa and OCI-AML3 cells demonstrated an increased sensitivity to inhibit cell growth by inducing DNA damage. Furthermore, HeLa cells incubated with splicing modulators have been shown to generate a dependency on ATR to counteract DNA damage, and the modulation of RNA splicing can make tumour cells more susceptible to ATR inhibitors. Human primary $CD34^+$ haematopoietic cells exposed to ATRi exhibit inhibited viability in a dose-dependent manner. The findings of Nguyen *et al* (59) revealed that the expression of U2AF1S34F in human primary $CD34^+$ haematopoietic cells induces a dramatic decrease in R-loop formation, making them vulnerable to ATR inhibition. These findings should be further investigated and validated in larger clinical trials.

IRAK4 inhibitors. A study on U2AF1 mutation and IRAK4 isoforms indicated that transfecting U2AF1 (S34F) into HEK293 IRAK4exon4 cells generated an increase in IRAK4 exon 4 products from the wild-type AAG splicing reporter compared with wild-type U2AF1. However, it did not affect IRAK4 exon 4 from the mutant TAG splicing reporter. This mutant U2AF1 was found to induce the expression of IRAK4, which plays a role in the activation of MAPK and NF-κB signalling to mediate the innate immune pathway (54). It was therefore suggested that inhibiting IRK4 activity may be a useful therapeutic strategy for diseases resulting from mutations in U2AF1 S34F.

IRAK1/4-Inh and CA-4948 are two distinct inhibitors targeting IRAK4. Structurally, the chemical compound of IRAK1/4-Inh is N-[1-(2-morpholin-4-ylethyl)-benzimidaz-zol-2-yl]-3-nitrobenzamide, while the clinical name of CA-4948 is emavusertib, formally named 6'-amino-N-[2-(4-morpholinyl) oxazolo [4, 5-b] pyridin-6-yl]-[2, 3'-bipyridine]-6-carboxamide (Fig. 5). The efficacy of both inhibitors was studied in K562 cells expressing U2AF1 S34F mutations. Cells incubated with IRAK1/4-Inh exhibited inhibited growth, which was dependent upon the deactivation of NF-κB signalling, while primary cells derived from AML or MDS patients with U2AF1 mutations incubated with CA-4948 underwent myeloid and erythroid differentiation. Taken together, these results strongly suggest that U2AF1 (S34F) malignant haematopoietic cells are sensitive to concentrations of IRAK4 inhibitors (89). Thus, inhibiting IRAK4 may be effective in diseases linked to U2AF1 S34F mutations.

Therapies targeting U2AF1 in clinical trials. At present, only a few relevant U2AF1 clinical studies have been included in clinical trials, namely, NCT04447651, NCT04278768 and NCT02060409. Of these clinical trials, NCT04278768

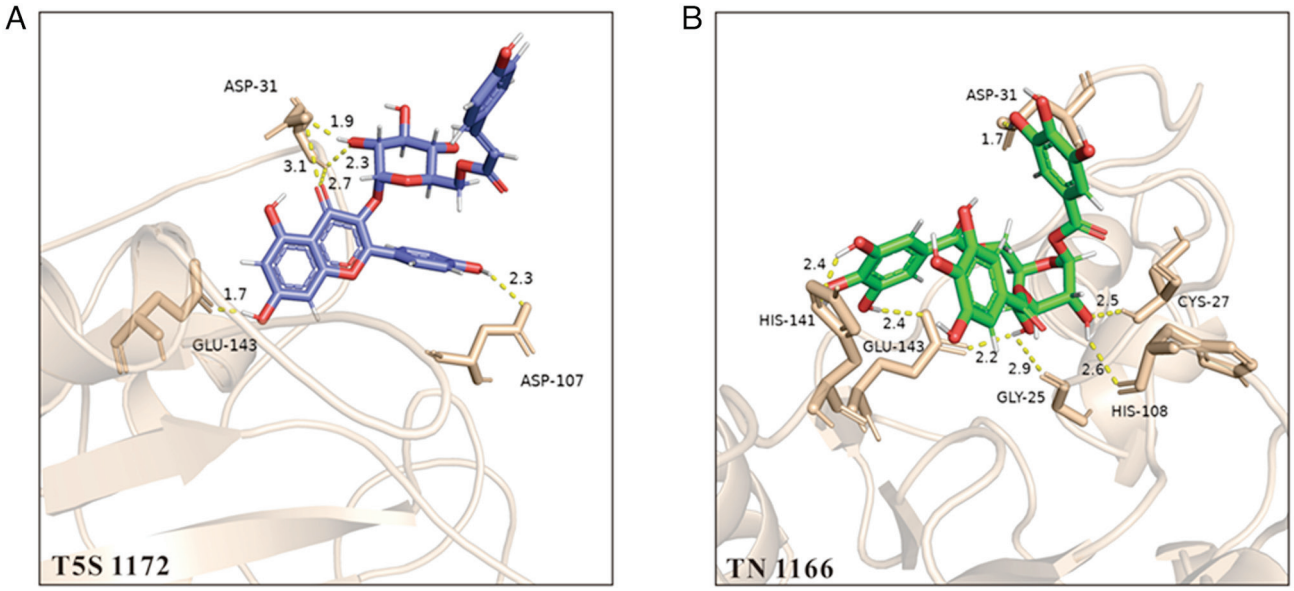

Figure 6. Scanning of natural product inhibitors. (A) Molecular docking model of tiliroside (T5S1172) bound to the U2AF1 protein structure, demonstrating the formation of hydrogen bonds between tiliroside and glutamic acid and aspartic acid molecules. (B) Model of 1, 3, 6-tri-O-galloylglucose (TN1166) docking with U2AF1, indicating the existence of hydrogen bonds between the molecules. U2AF1, U2 small nuclear RNA auxiliary factor 1.

was carried out in Phase 1 and Phase 2 for different trial diseases, while NCT02060409 was completed in clinical trials (Table II). These studies provide a promising outlook for the clinical application of U2AF1 in the future. Through rigorous study design and appropriate controls, this treatment may bring new opportunities for both diagnosis and therapy.

NCT04447651 aimed to explore the use of spliceosome mutational markers (PRISMMs) in the treatment of patients with metastatic solid tumours and haematological malignancies or lymphoma who may benefit from immunotherapy agents. NCT04278768 was a dose alteration trial of the monotherapy combination of CA-4948 and venetoclax or azacitidine in the treatment of AML or MDS patients with mutations in U2AF1, SRSF2, ZRSR2 and SF3B1. As a response to the increasing use of hypomethylating agents in MDS treatment, NCT02060409 investigated the prognostic impacts of U2AF1, SRSF, and ZRSR2 mutations after treatment with decitabine. Unfortunately, consistent prognostic relevance could not be established in this trial (90).

5. Discussion and perspectives

The spliceosome is a dynamic RNP complex that removes introns from nuclear pre-mRNAs and induces precise splicing at the 5' and 3' splice sites. This complex is composed of five small nuclear RNAs, U1, U2, U4, U5 and U6. Mutations in each of these subunits have been associated with various diseases. Notably, U2AF1, included in the U2 family, has a close correlation with haematological diseases. Mutations in U2AF1 have been observed in MDS, MDS-related AML, chronic myeloid leukaemia and PMF. The pathogenic mechanism of U2AF1 mutation is consideredto impair pre-mRNA splicing, thereby leading to DNA damage and loss of normal haematopoietic function (Table I) (91). Despite these findings, therapies targeting U2AF1 are scarce in clinical practice.

At present, the prognosis of U2AF1 mutations in malignant diseases, particularly in haematological diseases, remains to be fully elucidated. Most U2AF1 mutations have been observed to manifest as serine at site 34 being replaced by phenylalanine, along with glutamine at site 157 instead of arginine or proline. At sites 156 or 158, histidine can be replaced by arginine. As per previous relevant studies, only NSC194308 has been found to target U2AF1 directly, while other chemical compounds, such as sudemycin D6, ATR inhibitor, IRAK1/4-Inh and CA4948 (emavusertib), have been reported to have an indirect effect on U2AF1 mutations (92). Moreover, immunotherapies, such as anti-PD-1, anti-PD-L1 and anti-CTLA-4, may achieve favourable effects on U2AF1 mutations in MDS (93). It has been reported that IRAK4 may activate NF-kB in U2AF1 mutations, and thus, it is essential to assess the efficacy of NF-kB inhibitors in the therapy of U2AF1 mutations. Bortezomib or denosumab effectively broadly blocks the activity of NF-kB. Other NF-kB inhibitors known as IκBs are proteins that can mask nuclear localization signals of NF-κB to restrain its function. Given the association of NF-kB with U2AF1 mutations, it is necessary to explore the role of these inhibitors in therapies targeting U2AF1 mutations (94-96). In a study on oestrogen receptor-positive breast cancer, it was found that the silencing of U2AF1 in a zebrafish model might reduce the activity of various cell adhesion molecules, which further resulted in malignant differentiation. At the same time, natural products deserve their own stage, leveraging a computer-aided drug discovery online tool (http://cao.labshare.cn/drugrep/). A total of two natural products with favourable affinity were screened, both of them from Chinese herbal monomer extracts, named tiliroside and 1,3,6-tri-O-galloylglucose (Fig. 6). However, pre-silencing of U2AF1 RNAs may also promote new therapeutic options (97).

Given the limited availability of targeted therapies for spliceosomes, there is an urgent need to design more molecular targeted agents that act on cell proliferation and regulate tumour metabolism with the U2AF1 mutation representing a

significant target. Furthermore, further investigations into the targeting of spliceosome mutations should be carried out to explore the potential applications in the treatment of immunological or malignant diseases. Further studies are necessary to improve understanding of the implications of U2AF1 mutations.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Young Scientists Fund of the National Natural Science Foundation of China (grant no. 82204858) and the Sichuan Science and Technology Program (grant no. 2023NSFSC1761).

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

QN, YHL, JWL and RXL wrote or contributed to the writing of the manuscript. FRL, JHZ, LYZ, RXL and ZJY revised or contributed to revising the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ravi S, Schilder RJ and Kimball SR: Role of precursor mRNA splicing in nutrient-induced alterations in gene expression and metabolism. J Nutr 145: 841-846, 2015.
2. Saha K, Fernandez MM, Biswas T, Joseph S and Ghosh G: Discovery of a pre-mRNA structural scaffold as a contributor to the mammalian splicing code. Nucleic Acids Res 49: 7103-7121, 2021.
3. Scotti MM and Swanson MS: RNA mis-splicing in disease. Nat Rev Genet 17: 19-32, 2016.
4. Blijlevens M, Li J and van Beusechem VW: Biology of the mRNA splicing machinery and its dysregulation in cancer providing therapeutic opportunities. Int J Mol Sci 22: 5110, 2021.
5. Cléry A, Sinha R, Anczuków O, Corrionero A, Moursy A, Daubner GM, Valcárcel J, Krainer AR and Allain FH: Isolated pseudo-RNA-recognition motifs of SR proteins can regulate splicing using a noncanonical mode of RNA recognition. Proc Natl Acad Sci USA 110: E2802-E2811, 2013.
6. Moraleva AA, Deryabin AS, Rubtsov YP, Rubtsova MP and Dontsova OA: Eukaryotic ribosome biogenesis: The 60S subunit. Acta Naturae 14: 39-49, 2022.
7. Carrocci TJ and Neugebauer KM: Pre-mRNA splicing in the nuclear landscape. Cold Spring Harb Symp Quant Biol 84: 11-20, 2019.
8. Meyer K, Koester T and Staiger D: Pre-mRNA splicing in plants: In vivo functions of RNA-binding proteins implicated in the splicing process. Biomolecules 5: 1717-1740, 2015.
9. Sette C and Paronetto MP: Somatic mutations in core spliceosome components promote tumorigenesis and generate an exploitable vulnerability in human cancer. Cancers (Basel) 14: 1827, 2022.
10. Ilagan JO, Ramakrishnan A, Hayes B, Murphy ME, Zebari AS, Bradley P and Bradley RK: U2AF1 mutations alter splice site recognition in hematological malignancies. Genome Res 25: 14-26, 2015.
11. Yoshimi A and Abdel-Wahab O: Molecular pathways: Understanding and targeting mutant spliceosomal proteins. Clin Cancer Res 23: 336-341, 2017.
12. Fei DL, Zhen T, Durham B, Ferrarone J, Zhang T, Garrett L, Yoshimi A, Abdel-Wahab O, Bradley RK, Liu P and Varmus H: Impaired hematopoiesis and leukemia development in mice with a conditional knock-in allele of a mutant splicing factor gene U2af1. Proc Natl Acad Sci USA 115: E10437-E10446, 2018.
13. Chen S, Benbarche S and Abdel-Wahab O: Splicing factor gene mutations in hematologic malignancies. Blood 129: 1260-1269, 2017.
14. Ogawa S: Genetics of MDS. Blood 133: 1049-1059, 2019.
15. Inoue D, Bradley RK and Abdel-Wahab O: Spliceosomal gene mutations in myelodysplasia: Molecular links to clonal abnormalities of hematopoiesis. Genes Dev 30: 989-1001, 2016.
16. Taylor J and Lee SC: Mutations in spliceosome genes and therapeutic opportunities in myeloid malignancies. Genes Chromosomes Cancer 58: 889-902, 2019.
17. Przychodzen B, Jerez A, Guinta K, Sekeres MA, Padgett R, Maciejewski JP and Makishima H: Patterns of missplicing due to somatic U2AF1 mutations in myeloid neoplasms. Blood 122: 999-1006, 2013.
18. Madan V, Li J, Zhou S, Teoh WW, Han L, Meggendorfer M, Malcovati L, Cazzola M, Ogawa S, Haferlach T, *et al*: Distinct and convergent consequences of splice factor mutations in myelodysplastic syndromes. Am J Hematol 95: 133-143, 2020.
19. Zhou Z, Gong Q, Wang Y, Li M, Wang L, Ding H and Li P: The biological function and clinical significance of SF3B1 mutations in cancer. Biomark Res 8: 38, 2020.
20. Gupta G, Singh R, Kotasthane DS and Kotasthane VD: Myelodysplastic syndromes/neoplasms: Recent classification system based on World Health Organization classification of tumors-international agency for research on cancer for hematopoietic and lymphoid tissues. J Blood Med 1: 171-182, 2010.
21. Estey E, Hasserjian RP and Döhner H: Distinguishing AML from MDS: A fixed blast percentage may no longer be optimal. Blood 139: 323-332, 2022.
22. Chen J, Kao YR, Sun D, Todorova TI, Reynolds D, Narayanagari SR, Montagna C, Will B, Verma A and Steidl U: Myelodysplastic syndrome progression to acute myeloid leukemia at the stem cell level. Nat Med 25: 103-110, 2019.
23. Yu J, Li Y, Li T, Li Y, Xing H, Sun H, Sun L, Wan D, Liu Y, Xie X and Jiang Z: Gene mutational analysis by NGS and its clinical significance in patients with myelodysplastic syndrome and acute myeloid leukemia. Exp Hematol Oncol 9: 2, 2020.
24. Čolović N, Denčić-Fekete M, Peruničić M and Jurišić V: Clinical characteristics and treatment outcome of hypocellular acute myeloid leukemia based on WHO classification. Indian J Hematol Blood Transfus 36: 59-63, 2020.
25. Visconte V, O Nakashima M and J Rogers H: Mutations in Splicing Factor Genes In Myeloid Malignancies: Significance and impact on clinical features. Cancers (Basel) 11: 1844, 2019.
26. Follo MY, Pellagatti A, Ratti S, Ramazzotti G, Faenza I, Fiume R, Mongiorgi S, Suh PG, McCubrey JA, Manzoli L, *et al*: Recent advances in MDS mutation landscape: Splicing and signalling. Adv Biol Regul 75: 100673, 2020.
27. Brunner AM and Steensma DP: Targeting aberrant splicing in myelodysplastic syndromes: Biologic rationale and clinical opportunity. Hematol Oncol Clin North Am 34: 379-391, 2020.
28. Douet-Guilbert N, Soubise B, Bernard DG and Troadec MB: Cytogenetic and genetic abnormalities with diagnostic value in myelodysplastic syndromes (MDS): Focus on the pre-messenger RNA splicing process. Diagnostics (Basel) 12: 1658, 2022.
29. Dong Y, Li J, Zeng Z, Zhang X, Liang M, Yi H, Luo J and Li J: Growth retardation and congenital heart disease in a boy with a ring chromosome 6 of maternal origin. Mol Cytogenet 15: 9, 2022.

30. Li B, Zou D, Yang S, Ouyang G and Mu Q: Prognostic significance of U2AF1 mutations in myelodysplastic syndromes: A meta-analysis. J Int Med Res 48: 300060519891013, 2020.
31. Awada H, Thapa B and Visconte V: The genomics of myelodysplastic syndromes: Origins of disease evolution, biological pathways, and prognostic implications. Cells 9: 2512, 2020.
32. Esfahani MS, Lee LJ, Jeon YJ, Flynn RA, Stehr H, Hui AB, Ishisoko N, Kildebeck E, Newman AM, Bratman SV, *et al*: Functional significance of U2AF1 S34F mutations in lung adenocarcinomas. Nat Commun 10: 5712, 2019.
33. Kielkopf CL: Insights from structures of cancer-relevant pre-mRNA splicing factors. Curr Opin Genet Dev 48: 57-66, 2018.
34. Escobar-Hoyos L, Knorr K and Abdel-Wahab O: Aberrant RNA splicing in cancer. Annu Rev Cancer Biol 3: 167-185, 2019.
35. Biancon G, Joshi P, Zimmer JT, Hunck T, Gao Y, Lessard MD, Courchaine E, Barentine AE, Machyna M, Botti V, *et al*: Multi-omics profiling of U2AF1 mutants dissects pathogenic mechanisms affecting RNA granules in myeloid malignancies. bioRxiv: 2021.2004.2022.441020, 2021.
36. Martínez-Valiente C, Garcia-Ruiz C, Rosón B, Liquori A, González-Romero E, Fernández-González R, Gómez-Redondo I, Cervera J, Gutiérrez-Adán A and Sanjuan-Pla A: Aberrant alternative splicing in U2af1/Tet2 double mutant mice contributes to major hematological phenotypes. Int J Mol Sci 22: 6963, 2021.
37. Ivy KS and Brent Ferrell P Jr: Disordered immune regulation and its therapeutic targeting in myelodysplastic syndromes. Curr Hematol Malig Rep 13: 244-255, 2013.
38. Pellagatti A, Armstrong RN, Steeples V, Sharma E, Repapi E, Singh S, Sanchi A, Radujkovic A, Horn P, Dolatshad H, *et al*: Impact of spliceosome mutations on RNA splicing in myelodysplasia: dysregulated genes/pathways and clinical associations. Blood 132: 1225-1240, 2018.
39. Akef A, McGraw K, Cappell SD and Larson DR: Ribosome biogenesis is a downstream effector of the oncogenic U2AF1-S34F mutation. PLoS Biol 18: e3000920, 2020.
40. Xu Y and Ruggero D: The role of translation control in tumorigenesis and its therapeutic implications. Ann Rev Cancer Biol 4: 437-457, 2020.
41. Feliu N, Kohonen P, Ji J, Zhang Y, Karlsson HL, Palmberg L, Nyström A and Fadeel B: Next-generation sequencing reveals low-dose effects of cationic dendrimers in primary human bronchial epithelial cells. ACS Nano 9: 146-163, 2015.
42. Hallstrom TC, Mori S and Nevins JR: An E2F1-dependent gene expression program that determines the balance between proliferation and cell death. Cancer Cell 13: 11-22, 2008.
43. Wang H, Guo Y, Dong Z, Li T, Xie X, Wan D, Jiang Z, Yu J and Guo R: Differential U2AF1 mutation sites, burden and co-mutation genes can predict prognosis in patients with myelodysplastic syndrome. Sci Rep 10: 18622, 2020.
44. Huang FT, Sun J, Zhang L, He X, Zhu YH, Dong HJ, Wang HY, Zhu L, Zou JY, Huang JW and Li L: Role of SIRT1 in hematologic malignancies. J Zhejiang Univ Sci B 20: 391-398, 2019.
45. Carraway HE, Malkaram SA, Cen Y, Shatnawi A, Fan J, Ali HEA, Abd Elmageed ZY, Buttolph T, Denvir J, Primerano DA and Fandy TE: Activation of SIRT6 by DNA hypomethylating agents and clinical consequences on combination therapy in leukemia. Sci Rep 10: 10325, 2020.
46. Bhalla S and Gordon LI: Functional characterization of NAD dependent de-acetylases SIRT1 and SIRT2 in B-cell chronic lymphocytic leukemia (CLL). Cancer Biol Ther 17: 300-309, 2016.
47. Chen ML, Logan TD, Hochberg ML, Shelat SG, Yu X, Wilding GE, Tan W, Kujoth GC, Prolla TA, Selak MA, *et al*: Erythroid dysplasia, megaloblastic anemia, and impaired lymphopoiesis arising from mitochondrial dysfunction. Blood 114: 4045-4053, 2009.
48. Luo Y, Ma J and Lu W: The significance of mitochondrial dysfunction in cancer. Int J Mol Sci 21: 5598, 2020.
49. Park SM, Ou J, Chamberlain L, Simone TM, Yang H, Virbasius CM, Ali AM, Zhu LJ, Mukherjee S, Raza A and Green MR: U2AF35(S34F) promotes transformation by directing aberrant ATG7 pre-mRNA 3' end formation. Mol Cell 62: 479-490, 2016.
50. Zhao L, Cao J, Hu K, He X, Yun D, Tong T and Han L: Sirtuins and their biological relevance in aging and age-related diseases. Aging Dis 11: 927-945, 2020.
51. Bosch-Presegué L and Vaquero A: The dual role of sirtuins in cancer. Genes Cancer 2: 648-662, 2011.
52. Nakagawa MM, Chen H and Rathinam CV: Constitutive activation of NF-κB pathway in hematopoietic stem cells causes loss of quiescence and deregulated transcription factor networks. Front Cell Dev Biol 6: 143, 2018.
53. Balka KR and De Nardo D: Understanding early TLR signaling through the myddosome. J Leukoc Biol 105: 339-351, 2019.
54. Smith MA, Choudhary GS, Pellagatti A, Choi K, Bolanos LC, Bhagat TD, Gordon-Mitchell S, Von Ahrens D, Pradhan K, Steeples V, *et al*: U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. Nat Cell Biol 21: 640-650, 2019.
55. Pellagatti A and Boultwood J: SF3B1 mutant myelodysplastic syndrome: Recent advances. Adv Biol Regul 79: 100776, 2021.
56. Lee SC, North K, Kim E, Jang E, Obeng E, Lu SX, Liu B, Inoue D, Yoshimi A, Ki M, *et al*: Synthetic lethal and convergent biological effects of cancer-associated spliceosomal gene mutations. Cancer Cell 34: 225-241.e8, 2018.
57. Crossley MP, Bocek M and Cimprich KA: R-loops as cellular regulators and genomic threats. Mol Cell 73: 398-411, 2019.
58. Chen C, Zhou P, Zhang Z and Liu Y: U2AF1 mutation connects DNA damage to the alternative splicing of RAD51 in lung adenocarcinomas. Clin Exp Pharmacol Physiol 49: 740-747, 2022.
59. Nguyen HD, Leong WY, Li W, Reddy PNG, Sullivan JD, Walter MJ, Zou L and Graubert TA: Spliceosome mutations induce R loop-associated sensitivity to ATR inhibition in myelodysplastic syndromes. Cancer Res 78: 5363-5374, 2018.
60. Chen H, Libring S, Ruddraraju KV, Miao J, Solorio L, Zhang ZY and Wendt MK: SHP2 is a multifunctional therapeutic target in drug resistant metastatic breast cancer. Oncogene 39: 7166-7180, 2019.
61. Chen L, Chen JY, Huang YJ, Gu Y, Qiu J, Qian H, Shao C, Zhang X, Hu J, Li H, *et al*: The augmented R-loop is a unifying mechanism for myelodysplastic syndromes induced by high-risk splicing factor mutations. Mol Cell 69: 412-425.e6, 2018.
62. Boultwood J and Pellagatti A: The impact of spliceosome mutations in MDS. Hemasphere 3 (Suppl): S132-S134, 2019.
63. Vallapureddy RR, Mudireddy M, Penna D, Lasho TL, Finke CM, Hanson CA, Ketterling RP, Begna KH, Gangat N, Pardanani A and Tefferi A: Leukemic transformation among 1306 patients with primary myelofibrosis: Risk factors and development of a predictive model. Blood Cancer J 9: 12, 2019.
64. Tefferi A, Siragusa S, Hussein K, Schwager SM, Hanson CA, Pardanani A, Cervantes F and Passamonti F: Transfusion-dependency at presentation and its acquisition in the first year of diagnosis are both equally detrimental for survival in primary myelofibrosis-prognostic relevance is independent of IPSS or karyotype. Am J Hematol 85: 14-17, 2010.
65. Alhuraiji A, Naqvi K, Huh YO, Ho C, Verstovsek S and Bose P: Acute lymphoblastic leukemia secondary to myeloproliferative neoplasms or after lenalidomide exposure. Clin Case Rep 6: 155-161, 2017.
66. Tefferi A, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hanson CH, Maffioli M, Caramazza D, Passamonti F and Pardanani A: CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: Clinical, cytogenetic and molecular comparisons. Leukemia 28: 1472-1477, 2014.
67. Chaligné R, James C, Tonetti C, Besancenot R, Le Couédic JP, Fava F, Mazurier F, Godin I, Maloum K, Larbret F, *et al*: Evidence for MPL W515L/K mutations in hematopoietic stem cells in primitive myelofibrosis. Blood 110: 3735-3743, 2007.
68. Tefferi A, Finke CM, Lasho TL, Hanson CA, Ketterling RP, Gangat N and Pardanani A: U2AF1 mutation types in primary myelofibrosis: Phenotypic and prognostic distinctions. Leukemia 32: 2274-2278, 2018.
69. Tefferi A, Mudireddy M, Finke CM, Nicolosi M, Lasho TL, Hanson CA, Patnaik MM, Pardanani A and Gangat N: U2AF1 mutation variants in myelodysplastic syndromes and their clinical correlates. Am J Hematol 93: E146-E148, 2018.
70. Wu SJ, Tang JL, Lin CT, Kuo YY, Li LY, Tseng MH, Huang CF, Lai YJ, Lee FY, Liu MC, *et al*: Clinical implications of U2AF1 mutation in patients with myelodysplastic syndrome and its stability during disease progression. Am J Hematol 88: E277-E282, 2013.
71. Patnaik MM and Tefferi A: Chronic myelomonocytic leukemia: 2020 Update on diagnosis, risk stratification and management. Am J Hematol 95: 97-115, 2020.
72. Machherndl-Spandl S, Jäger E, Barna A, Gurbisz M, Marschon R, Graf T, Graf E, Geissler C, Hoermann G, Nösslinger T, *et al*: Impact of age on the cumulative risk of transformation in patients with chronic myelomonocytic leukaemia. Eur J Haematol 107: 265-274, 2021.

73. Patnaik MM, Lasho TL, Finke CM, Hanson CA, Hodnefield JM, Knudson RA, Ketterling RP, Pardanani A and Tefferi A: Spliceosome mutations involving SRSF2, SF3B1, and U2AF35 in chronic myelomonocytic leukemia: Prevalence, clinical correlates, and prognostic relevance. Am J Hematol 88: 201-206, 2013.
74. Patnaik MM and Tefferi A: Cytogenetic and molecular abnormalities in chronic myelomonocytic leukemia. Blood Cancer J 6: e393, 2016.
75. McClure RF, Ewalt MD, Crow J, Temple-Smolkin RL, Pullambhatla M, Sargent R and Kim AS: Clinical significance of DNA variants in chronic myeloid neoplasms: A report of the association for molecular pathology. J Mol Diagn 20: 717-737, 2018.
76. Grever MR, Abdel-Wahab O, Andritsos LA, Banerji V, Barrientos J, Blachly JS, Call TG, Catovsky D, Dearden C, Demeter J, *et al*: Consensus guidelines for the diagnosis and management of patients with classic hairy cell leukemia. Blood 129: 553-560, 2017.
77. Kreitman RJ: Hairy cell leukemia: Present and future directions. Leuk Lymphoma 60: 2869-2879, 2019.
78. Durham BH, Getta B, Dietrich S, Taylor J, Won H, Bogenberger JM, Scott S, Kim E, Chung YR, Chung SS, *et al*: Genomic analysis of hairy cell leukemia identifies novel recurrent genetic alterations. Blood 130: 1644-1648, 2017.
79. Rahman MA, Krainer AR and Abdel-Wahab O: SnapShot: Splicing alterations in cancer. Cell 180: 208-208.e1, 2020.
80. Fei DL, Motowski H, Chatrikhi R, Prasad S, Yu J, Gao S, Kielkopf CL, Bradley RK and Varmus H: Wild-type U2AF1 antagonizes the splicing program characteristic of U2AF1-mutant tumors and is required for cell survival. PLOS Genetics 12: e1006384, 2016.
81. Sanchez A, El Ouardi D, Houfaf Khoufaf FZ, Idrissou M, Boisnier T, Penault-Llorca F, Bignon YJ, Guy L and Bernard-Gallon D: Role of JMJD3 demethylase and its inhibitor GSK-J4 in regulation of MGMT, TRA2A, RPS6KA2, and U2AF1 genes in prostate cancer cell lines. OMICS 24: 505-507, 2020.
82. Cao H, Wang D, Gao R, Chen L and Feng Y: Down regulation of U2AF1 promotes ARV7 splicing and prostate cancer progression. Biochem Biophys Res Commun 541: 56-62, 2021.
83. El Ouardi D, Idrissou M, Sanchez A, Penault-Llorca F, Bignon YJ, Guy L and Bernard-Gallon D: The inhibition of the histone methyltransferase EZH2 by DZNEP or SiRNA demonstrates its involvement in MGMT, TRA2A, RPS6KA2, and U2AF1 gene regulation in prostate cancer. OMICS 24: 116-118, 2020.
84. Je EM, Yoo NJ, Kim YJ, Kim MS and Lee SH: Mutational analysis of splicing machinery genes SF3B1, U2AF1 and SRSF2 in myelodysplasia and other common tumors. Int J Cancer 133: 260-265, 2013.
85. Chatrikhi R, Feeney CF, Pulvino MJ, Alachouzos G, MacRae AJ, Falls Z, Rai S, Brennessel WW, Jenkins JL, Walter MJ, *et al*: A synthetic small molecule stalls pre-mRNA splicing by promoting an early-stage U2AF2-RNA complex. Cell Chem Biol 28: 1145-1157.e6, 2021.
86. Lagisetti C, Palacios G, Goronga T, Freeman B, Caufield W and Webb TR: Optimization of antitumor modulators of pre-mRNA splicing. J Med Chem 56: 10033-10044, 2013.
87. Shirai CL, White BS, Tripathi M, Tapia R, Ley JN, Ndonwi M, Kim S, Shao J, Carver A, Saez B, *et al*: Mutant U2AF1-expressing cells are sensitive to pharmacological modulation of the spliceosome. Nat Commun 8: 14060, 2017.
88. Middleton MR, Dean E, Evans TRJ, Shapiro GI, Pollard J, Hendriks BS, Falk M, Diaz-Padilla I and Plummer R: Phase 1 study of the ATR inhibitor berzosertib (formerly M6620, VX-970) combined with gemcitabine ± cisplatin in patients with advanced solid tumours. Br J Cancer 125: 510-519, 2021.
89. Powers JP, Li S, Jaen JC, Liu J, Walker NP, Wang Z and Wesche H: Discovery and initial SAR of inhibitors of interleukin-1 receptor-associated kinase-4. Bioorg Med Chem Lett 16: 2842-2845, 2006.
90. Thol F, Kade S, Schlarmann C, Löffeld P, Morgan M, Krauter J, Wlodarski MW, Kölking B, Wichmann M, Görlich K, *et al*: Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood 119: 3578-3584, 2012.
91. Griffin C and Saint-Jeannet JP: Spliceosomopathies: Diseases and mechanisms. Dev Dyn 249: 1038-1046, 2020.
92. Zhao Y, Cai W, Hua Y, Yang X and Zhou J: The biological and clinical consequences of RNA splicing factor U2AF1 mutation in myeloid malignancies. Cancers (Basel) 14: 4406, 2022.
93. Lee P, Yim R, Yung Y, Chu HT, Yip PK and Gill H: Molecular targeted therapy and immunotherapy for myelodysplastic syndrome. Int J Mol Sci 22: 10232, 2021.
94. Jacobs MD and Harrison SC: Structure of an IkappaBalpha/NF-kappaB complex. Cell 95: 749-758, 1998.
95. Raedler L: Velcade (bortezomib) receives 2 new FDA indications: For retreatment of patients with multiple myeloma and for first-line treatment of patients with mantle-cell lymphoma. Am Health Drug Benefits 8: 135-140, 2015.
96. Hamdy NA: Denosumab: RANKL inhibition in the management of bone loss. Drugs Today (Barc) 44: 7-21, 2008.
97. Vazquez Rodriguez G, Abrahamsson A, Turkina MV and Dabrosin C: Lysine in combination with estradiol promote dissemination of estrogen receptor positive breast cancer via upregulation of U2AF1 and RPN2 proteins. Front Oncol 10: 598684, 2020.