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Germline and Somatic Defects in *DDX41* and its Impact on Myeloid Neoplasms

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Abstract

Purpose of Review—While *DDX41* mutation (m) is one of the most prevalent predisposition genes in adult myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML), most patients do not always present with a family history of MDS/AML. In this review, we will be highlighting epidemiological data on *DDX41*m, roles of *DDX41* in oncogenesis, mechanisms of clonal evolution with somatic *DDX41*m, and clinical phenotypes and management of MDS/AML in patients harboring *DDX41*m.

Recent Findings—*DDX41* encodes a DEAD-box helicase protein that is considered essential for cell growth and viability. High incidence of myeloid malignancies and other cancers in patients bearing *DDX41*m suggests that defects in *DDX41* lead to loss of a tumor suppressor function, likely related to activities in RNA splicing and processing pathways. Seventy percent of cancer cases with *DDX41*m are associated with MDS/AML alone. More than 65% of familial cases harbor heterozygous germline frameshift mutations, of which p.D140Gfs*2 is the most common. A somatic *DDX41*m of the second allele is acquired in 70% of cases, leading to hematological malignancy. Myeloid neoplasms with *DDX41*m are typically characterized by long latency, high-risk disease at presentation with normal cytogenetics and without any additional molecular markers. Recent reports suggests that a subgroup of these patients have an indolent clinical course and have a better long-term survival compared to favorable or intermediate risk AML.

Summary—Distinct clinical/pathologic features and favorable outcomes in MDS/AML highlight the need for standardized classification and gene specific guidelines that could assist in management decisions in patients with *DDX41*m.

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Declarations

Human and Animal Rights and Informed Consent. This article does not contain any studies with human or animal subjects performed by any of the authors.

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Keywords

DDX41 ; AML; MDS; Familial MDS/leukemia

Introduction

Germline mutations that predispose to hereditary cancers have been reported in several genes.[1] The World Health Organization 2016 classification recognizes hematological malignancies with germline predisposition as a distinct subgroup.[2] Identifying and risk stratifying germline mutations in hematological malignancies is important in adapting risk stratified treatment algorithms.[3] DEAD/H-box helicase 41 gene (*DDX41*) located on chromosome 5q35 is thought to be a tumor suppressor gene involved in the splicing of pre-mRNAs and processing of ribosomal RNA. Defects in *DDX41* lead to loss of its tumor suppressor function and in experimental knockout systems it has been shown that *DDX41* may participate in the development of myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). [4, 5] Germline *DDX41* lesions define a subset of hereditary myeloid neoplasms that are associated with long latency, high-risk MDS/AML at presentation, strong male bias, and normal cytogenetics without any additional molecular markers. At the time of hematologic malignancy, a somatic *DDX41* mutation of the second allele, commonly p.R525H variant, can be acquired in more than half of the cases. Some studies suggest that while p.R525H is a risk factor for progression to AML, other somatic variants (p.T227M, p.P321L, and p.G530D) could link to less common *DDX41*-related hematologic diseases, such as aplastic anemia.[4,6–8] The pro-leukemogenic properties of *DDX41* lesions are supported by the presence of somatic mutations and lack of common MDS/AML associated cytogenetic or molecular abnormalities.[9] Due to its association with familial predisposition syndromes, detection of *DDX41* mutation should be followed by genetic counseling and evaluation of family members. In this review, we will highlight the epidemiological data on *DDX41* mutation, roles of *DDX41* in oncogenesis, mechanisms of clonal evolution with somatic *DDX41* mutation, clinical phenotypes of neoplasm associated with *DDX41*, analytical tools that can be used to analyze *DDX41* variants of undetermined significance (VUS), and clinical management of MDS/AML patients harboring *DDX41* mutation.

Epidemiologic Data and Clinical Phenotype of Neoplasm Associated with *DDX41* Variants

Traditionally, evaluation of only affected families was conducted to identify familial myeloid neoplasms. Increased utilization of whole exome sequencing facilitated efficient identification of families affected with germline predisposition alleles.[4, 6] Familial inheritance of *DDX41*m is reported in 0.5 to 4% of adult AML/MDS cohorts compared to prevalence of around 5% of all other familial syndromes associated with MDS/AML. [4, 9, 10] While germline *DDX41* mutations predispose to a variety of hematological malignancies including MDS, AML, chronic myeloid leukemia (CML), and lymphomas, 70% of cases are associated with MDS/AML alone.[10] According to a most recent systemic review by Wang et al., 181 probable germline variants have been confirmed by cultured skin fibroblasts, oral epithelial cells, clear transmitting pedigree, and others. *DDX41* missense variants composed the largest part, followed by frameshift, nonsense, splicing, and in-frame deletion/duplication in patients with myeloid neoplasms.[8] The top

five germline variants identified were p.D140fs, p.M1I, p.V152G, p.Y259C, and p.A500fs. They showed an apparent difference in distribution in different countries: p.M1I and p.D140fs in Europe and the USA and p.V152G, p.Y259C, and p.A500fs in Asia. Many sequencing studies revealing the incidence of germline *DDX41*m in general MDS/AML patient cohorts have been published in recent years, and the combined analysis of these reports informs a general consensus of mutational spectra, patient characteristics, and disease course.[3, 6, 10–12] As an example, Lewinsohn et al. evaluated 289 families with suspected hereditary myeloid malignancies wherein heterozygous germline *DDX41*m was identified in 9 new families (3%) who had no other detectable predisposition allele. [12] Among these, 3 families carried the recurrent p.D140Gfs*2 mutation; 1 family carried a germline p.R525H (c.1574G.A) mutation, and 5 carried other novel mutations (p.M1I, p.G530D, p.R164W). Families carrying the germline *DDX41* p.D140Gfs*2 or p.M1I demonstrated late-onset MDS or AML; however, one individual with *DDX41* p.M1I developed chronic myeloid leukemia and another developed both AML and non-Hodgkin lymphoma (NHL). In the same report, 5/5 affected individuals with NHL, Hodgkin lymphoma, or multiple myeloma carried the p.R164W variant. Families with p.R52H and p.G530D had high penetrance of MDS/AML and younger age of onset. In Mayo Clinic, p.M1I was the most common germline variant in a cohort of 33 *DDX41* mutated patients observed with myeloid neoplasms.[9] This cohort and many others have also demonstrated male predominance, normal cytogenetics, and less frequent association with family history of solid tumors.[9] In another study from France on 43 *DDX41* mutated myeloid malignancies patients, in addition to variants mentioned above, a novel p.G173R germline *DDX41* variant was discovered in 6 patients with similar biological and clinical features.[3] In several studies, the most common somatic variant was p.R525H; other frequent somatic variants were p.T227M, p.P321L, and p.G530D. In a recent systemic review, Wang et al. compared patients carrying p.R525H and other somatic variants. Somatic p.R525H was significantly associated with p. M1I germline variants. Moreover, p.R525H somatic mutation was significantly associated with risk of progression to AML. Other somatic variants suggestive of possible link to less common *DDX41*-related hematologic diseases, such as aplastic anemia. Clinical phenotype and characteristics of hematological malignancies associated with *DDX41* germline variants are outlined in Table 1.

Function of *DDX41* and its Role in Oncogenesis

DDX41 is a member of the DEAD-box family of RNA helicases, a group of at least 38 proteins that share conserved nucleic acid binding and ATP hydrolysis motifs forming a core helicase domain.[13] These proteins function in a diverse array of cellular activities including transcription, RNA splicing, translation initiation, and innate immune sensing.[13] Initial studies described DDX41 as an innate immune sensor functioning upstream of the STING pathway in innate immune effector cells.[14, 15] In dendritic cells, DDX41 is required for interferon release in response to infection by DNA viruses such as HSV-1[14] (Fig. 1). Subsequent studies showed that DDX41 binds several viral and bacterial by-products in the cytosol including double-stranded DNA and cyclic di-nucleotides, leading to innate immune activation.[15] Upon ligand binding, DDX41 interacts physically with the STING protein, inducing its activation of TBK1 which then activates the IRF3 and IRF7 transcription factors to induce interferon gene expression.[14–16] The DDX41-STING

interaction is dependent on Bruton's tyrosine kinase (BTK), and DDX41 is a direct target of BTK phosphorylation[16] (Fig. 1). Mice with dendritic cell-specific knockout of *DDX41* are more easily infected with murine leukemia virus (MLV), suggesting that loss of DDX41-mediated sensing and innate immune pathway activation plays an important role in the anti-viral response. [17, 18] Despite these findings, there is no reported immune deficiency in *DDX41* mutation carriers, likely because one functional copy of *DDX41* is sufficient to maintain activity of this pathway.

Related to its role in anti-retroviral innate immune defense mechanisms, DDX41 was found to preferentially bind RNA:DNA hybrids produced during retroviral reverse transcription compared to dsDNA.[18] Thus, it is thought that DDX41 binds the earliest intermediates of reverse transcription, leading to early STING activation, whereas other dsDNA sensors recognize dsDNA produced in subsequent steps of viral replication.[18] Beyond its antiviral functions, DDX41 can also bind naturally occurring RNA:DNA hybrid structures in the nucleus, called R-loops[19, 20] (Fig. 1). These are 3-stranded structures of RNA:DNA hybrid and ssDNA that occur during transcription and play regulatory roles in gene regulation. R-loops are susceptible to double-strand breaks and other DNA damage and are thus associated with genome instability. A study of *Ddx41* knockdown in zebrafish embryos showed that loss of *Ddx41* caused HSPC to accumulate R-loops, to have elevated DNA damage and innate immune activation, and to increase in number through increased developmental production.[19] An unbiased screen for R-loop binding proteins in mammalian cells identified DDX41 as a critical regulator of R-loops at sites of transcription initiation.[20] By preventing or unraveling R-loops, DDX41 functions to reduce the overall R-loop burden on a cell, reduce DNA damage, and suppress innate immune activation. [19–21] These findings of DDX41 as a suppressor of innate immune activation through R-loop regulation is counter-intuitive but not necessarily incompatible with its role as a cytosolic innate immune sensor. Cell type and cellular compartmentalization are likely critical determinants of the effect of DDX41 protein on innate immune pathway activation.

When germline *DDX41* mutations were initially discovered in families with high rates of myeloid malignancies, Polprasert and colleagues sought to identify novel functions of DDX41 to explain its role in oncogenesis.[22] They conducted a screen for DDX41-interacting proteins and identified multiple components of the core mRNA splicing machinery. Analysis of mRNA splicing in AML blasts from *DDX41*-mutant patients compared to patients without a *DDX41* mutation revealed that mis-splicing events were prevalent when *DDX41* is mutated (Fig. 1). Since splicing factor mutations are common in myeloid malignancies, it is likely that global or locus-specific missplicing contributes to pathogenesis of these cancers. In particular, an exon skipping event in the gene *ZMYM2* was significantly increased in *DDX41*-mutant AML samples compared to controls, and this difference was recapitulated in experimental models. However, the role of *ZMYM2* or other specific mis-splicing events downstream of *DDX41* mutations in promoting myeloid transformation remains poorly understood.

To determine the role of *DDX41* in normal hematopoiesis, Chlon and colleagues recently reported the generation of *DDX41* knockout and *DDX41*-R525H mutant mice, which model the most common somatic *DDX41* mutation observed in patients.[23] Complete knockout

of *DDX41* is embryonic lethal, indicating that it is an essential gene and confirming the likely reason why patients with biallelic germline *DDX41* mutations have not been observed. Inducible excision of the *DDX41* alleles in hematopoietic progenitor cells caused cell cycle arrest and apoptosis, indicating that *DDX41* is required for hematopoietic progenitor cell survival and proliferation. Additionally, they showed that the R525H mutant of DDX41 is lacking in the essential DDX41 function since $DDX41^{R525H/-}$ progenitor cells also failed to survive and proliferate. Mechanistically, DDX41 was found to be required for snoRNA processing and ribosome biogenesis (Fig. 1), explaining the low protein translation rates observed in *DDX41*-deficient hematopoietic progenitor cells. A study of human CD34 + hematopoietic progenitors where the $DDX41^{R525H}$ mutant was over-expressed also demonstrated that *DDX41* mutations induce ribosome biogenesis defects.[24] Analysis of transcriptomic data from MDS patient cohorts found evidence of a similar snoRNA processing defect in bone marrow cells from patients bearing $DDX41^{R525H}$ mutations, suggesting that snoRNA dysregulation is conserved in humans and relevant to MDS. The precise molecular contribution of DDX41 to the complex process of snoRNA processing remains to be defined, but it is possible that there is overlap with its function in mRNA splicing since splicing is a required step in snoRNA synthesis. Other gene mutations affecting ribosome biogenesis are common in MDS, including inherited (Diamond-Blackfan anemia) or acquired (del5q MDS) heterozygous loss of ribosomal subunit genes. Importantly, snoRNA and ribosome defects were not observed in cells with heterozygous mutations in *DDX41*, and thus the cause of hematologic disease in germline *DDX41* patients where an acquired *DDX41* mutation is not observed remains to be elucidated. Furthermore, the mechanism of clonal expansion when germline and acquired *DDX41* mutations are present is poorly understood since this combination of mutations caused cell cycle arrest and apoptosis in murine hematopoietic progenitor cells.[23]

Analytic Tools That Can Be Used or That Are in Development for Assessing Variants of Undetermined Significance

Germline mutations in *DDX41* that predispose to myeloid malignancy are thought to be loss-of-function mutations since the most prevalent mutations are nonsense or start site mutations that are expected to cause loss of full-length protein expression.[22] However, more than half of patients present with missense and splice site germline mutations, and the effect of these mutations on *DDX41* function is typically not known.[25] To determine the effect of these mutations on DDX41 expression and function, an assay to assess the *DDX41* activity derived from these gene variants is necessary. The most tractable and disease-relevant assays for gene activity are cell-based assays where the activity of the gene can be measured by a change in cell phenotype or a reporter assay. Since *DDX41* is required for cell survival and proliferation, *DDX41*-deficient cell lines cannot be maintained long term. Thus, the acquisition of *DDX41*-deficient or mutant cells necessitates transient experimental systems. These could include RNAi-mediated knockdown approaches, transient knockout, mutation by CRISPR, or excision of floxed alleles by inducible Cre-recombinase proteins. The latter provides the most complete removal of *DDX41* protein and thus enables complementation studies whereby cells with excision at both *DDX41* alleles require expression of an exogenous *DDX41* cDNA to rescue their proliferation and survival. To achieve a cell line model with inducible excision of floxed *DDX41* alleles, $DDX41^{flox/flox}$;

Rosa-CreERT2 hematopoietic progenitor cells were immortalized by expression of the MLL-AF9 transgene. These cells grow indefinitely in culture until they are treated with 4-hydroxy-tamoxifen, which activates the CreERT2 protein, causing recombination at both *DDX41* alleles, loss of DDX41 protein, and cell death.[23] The growth of these cells can be rescued by exogenous expression of wild-type *DDX41* from a lentivirus. However, expression of *DDX41*^{R525H} mutant by the same approach does not rescue the growth of these cells, demonstrating that the R525H mutation causes loss of the critical *DDX41* function. In a similar fashion, *DDX41* VUS could be tested in this assay by generating the cDNA encoded by the VUS in a lentivirus and testing its ability to complement the loss of *DDX41* in these cells. For splicing or intronic mutations, the expressed transcript in the lentivirus could be engineered to include the wild-type or mutant intron to test for the effect of altered splicing on the expression and activity of the VUS gene compared to control. One limitation is that this complementation assay only tests for loss of the cell essential *DDX41* function and does not indicate whether any non-essential DDX41 functions, such as innate immune sensing, are affected by the mutation. Reporter assays for interferon gene activation could be used for this purpose, although this would require generating differentiated myeloid cells that would not undergo cell death when *DDX41* expression is lost.

Mechanisms of Clonal Evolution with Somatic *DDX41* and Clonal Hematopoiesis Mutations

Emerging patient data suggest that heterozygous *DDX41* mutations cause cytopenias of undetermined significance in some patients prior to developing MDS or other myeloid malignancies.[26] Whether these cytopenias are caused by clonal stem and progenitor cells and whether cooperating mutations are already present in the stem cell pool of these patients remain to be determined. Sequencing studies on *DDX41* mutated MDS/AML patient cohorts have indicated that many common CHIP mutations, such as *DNMT3A* (10.9%), *ASXL1* (26.4%), *TET2* (13.2%), and *TP53* (22.5%), are recurrent in *DDX41*-mutant MDS/AML but are not found in all patients.[3, 10, 22, 26, 27] CHIP mutations are present at similar frequencies in *DDX41* mutated patients compared to general MDS/AML cohorts, with *ASXL1* being the most prevalent of the CHIP mutations found in 18–28% of patients.[3, 10, 25] Thus, it seems that CHIP mutations do promote clonal hematopoiesis in patients bearing germline *DDX41* mutations, and they likely play a role in the development of malignancy but may not be essential for disease development. In contrast, somatic *DDX41* mutations are present in approximately 70% of germline DDX41 patients presenting with MDS/AML, which is far greater than the prevalence of somatic *DDX41* mutations in general MDS/ AML cohorts (1–2%).[25] Thus, it appears that there is a particular selection for these mutations in the stem cell pool of germline *DDX41* patients. Paradoxically, recent studies in mice and human cells have demonstrated that the most common somatic mutation *DDX41*-R525H causes loss of DDX41 function and, when combined with a loss-of-expression mutation as is often the case with germline mutations, causes hematopoietic progenitor cell proliferation defects. Thus, the mechanism of selection for the somatic *DDX41* mutation when it causes cell cycle arrest and apoptosis remains to be determined. One theory is that because HSC have lower levels of protein translation than progenitor cells and their stemness depends on low translation,[28] then the somatic *DDX41* mutations are tolerated or even selected for in the HSC and then cause hematopoietic defects due to their inability to proliferate as progenitors.[29] In this way, the somatic *DDX41* mutation could cause disease

merely by limiting the number of functioning HSC, leading to hematopoietic failure. This theory is supported by the hypocellularity of these MDS patients' bone marrow, in contrast to the hypercellularity that is typical of adult MDS. Alternatively, the death of HSPC bearing biallelic *DDX41* mutations when they are activated to proliferate may contribute to disease pathogenesis through cell extrinsic effects on the rest of the bone marrow stem and progenitor cells.[23] The available data indicate that cell intrinsic and extrinsic effects of the somatic *DDX41* mutation play a role in disease pathogenesis.

When a somatic *DDX41* mutation is observed in the context of a germline *DDX41* predisposition syndrome, it typically has a low variant allele frequency (median of 10%), indicating that these mutations promote disease progression but are not likely present in the most proliferative disease clones. Recent clinical reports of donor cell leukemias arising in related hematopoietic stem cell transplant recipientdonor pairs with germline *DDX41* mutations are illustrative of a potential role of the somatic *DDX41*-R525H mutation as a minor clone in *DDX41* mutant leukemia.[30–32] In one instance, the recipient was treated for MDS-EB2 by bone marrow transplantation with a sibling as the donor.[30] The initial MDS had a somatic *DDX41* mutation with a VAF of 7.9%. Seventeen months post-transplant, he developed MDS-EB2 that subsequently developed into AML. The donor cell leukemia had a *DDX41*-R525H VAF of 6.7%, whereas sequencing of the donor bone marrow sample prior to transplant revealed a VAF of just 0.4%, indicating that the R525H mutant clone expanded after transplantation, but still was not the dominant clone in the leukemia. No other common clonal hematopoiesis gene mutations were observed in either of the disease samples. Thus, it is likely that the R525H mutant clone played a critical role in disease progression but is not likely present in the dominant, most rapidly proliferating clone. Further research to determine the method of selection of the somatic *DDX41* mutation and the cell extrinsic effects of these mutant HSC on the rest of the bone marrow is necessary to elucidate their role in disease pathogenesis.

Management and Outcome of Patients with *DDX41* Mutated MDS/AML

As noted above, typical *DDX41* associated myeloid neoplasms (MDS, AML, and CMML), presents in the fifth through eight decades of life, and may not have an obvious familial history at diagnosis. At this point in time, there is no consensus guidelines established for management of *DDX41* mutated MDS/AML. Patients eligible for intensive chemotherapy are treated with standard of care therapy followed by allogeneic hematopoietic stem cell transplant, while elderly patients are treated with less intensive, hypomethylating agent-based therapies.[3] Recent reports suggests that a subgroup of these patients have a indolent clinical course, relatively higher complete remission rates (70–80%), and better long-term survival compared to favorable or intermediate risk AML.[33, 34] In Mayo Clinic cohort of 33 *DDX41* mutated high-risk MDS/AML, 77% of patients with AML had 100% complete remission (CR) when treated with induction chemotherapy or hypomethylating agents (HMA) plus venetoclax regimen.[9] The median overall survival (OS) was not reached; 2-year OS was 86%. In the same cohort, 12 (41%) patients received hematopoietic stem cell transplantation (HSCT), and there was no difference in 2-year OS between patients who received HSCT vs no HSCT. When survival outcome of mutated *DDX41* AML was compared to the core-binding factor [t (8;21)] AML, significantly superior outcome was

observed in mutated *DDX41* AML (2-year OS, 100% vs 51%; $p = 0.024$). Similarly, in a series of 28 *DDX41* mutated patients from France, 20 patients had high-risk MDS/AML, 9 were treated with intensive chemotherapy, and 11 were treated with azacitidine with an overall response rate of 100% and 73%, respectively, with a median overall survival of 5.2 years.[3]

Polprasert et al. demonstrated efficacy of lenalidomide in identical twin brothers with a germline *DDX41* variant (c.T1187C; p.I396T) diagnosed with MDS (refractory anemia with multilineage dysplasia).[6] In another series ($n = 9$) of non-del (5q) MDS patients with heterozygous *DDX41* mutations or deletions, response rate to lenalidomide was 57% with odd ratio (OR) of 1.3.[35] Similarly, in another study of 139 non-del (5q) MDS patients, responsiveness to lenalidomide was higher in *DDX41* mutated MDS patients carrying normal cytogenetics.[36] The clinical efficacy of lenalidomide in *DDX41* mutated myeloid neoplasm needs to be further evaluated prospectively in a larger cohort of patients.

Conclusion

The relatively high variant frequency in MDS/AML patients compared to other hereditary hematological malignancies makes *DDX41* the most common predisposition gene in adults with MDS/AML. Reports of distinct clinical/pathologic features and favorable outcomes in MDS/AML as a specific entity highlight the need for standardized classification and gene specific guidelines that could further assist clinical management decisions.

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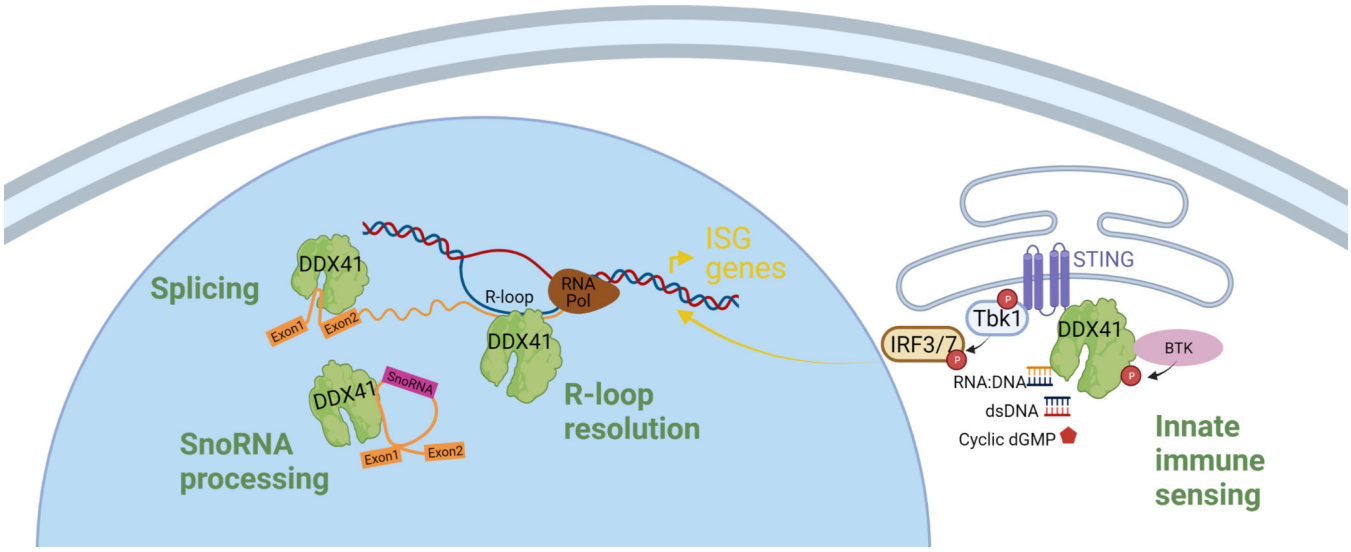

Fig. 1.

Functions of DDX41 protein. DDX41 functions as an innate immune sensor in mature myeloid cells. It binds to various ligands, and then interacts with STING to induce interferon signaling though TBK1-IRF3/7 activation. DDX41 also functions in pre-mRNA splicing, snoRNA processing, and R-loop resolution in the nucleus

Table 1.

Clinical phenotype and characteristics of hematological malignancies associated with DDX41 variants

Author	DDX41 variant (Germline)	DDX41 variant (Somatic)	Hematological Malignancy	Age of onset (decade/years)	Family History of HM
Polprasert and others [4,6,12]	p.D140Gfs	-	MDS/AML	60's	Yes
Polprasert[6]	p.Q52fs	P.A225D	AML	70's	Yes
Polprasert[6]	p.I396T	p.R525H	MDS (RCMD)	60's	Yes
Polprasert[6]	p.M155I	-	MDS-EB1	70's	Yes
Lewinsohn and others [3,4,6,9,12]	p.M1I	-	MDS/AML/CML/NHL	60's	Yes
Lewinsohn and others [3,12]	p.R164W	-	HL/NHL/MM	50's	Yes
Sebert [3]	p.G173R	p.R525H	MDS	70's	No
Sebert [3]	p.G173R	p.T227M	AA→MDS-EB1	60's	No
Sebert [3]	p.Q41X	p.R525H/ p.A225V	MDS-EB1	70's	No
Sebert [3]	p.K106X	-	CMML	70's	Yes
Sebert [3]	p.E114fs	p.E345D	MDS-EB2	80's	No
Sebert [3]	p.K331del	p.T227M	MDS/MPN	60's	No
Sebert [3]	p.K331del	p.R525H	MDS-EB2/AML	60's	Yes
Sebert [3]	p.R479Q	-	MDS/AML	60's	No
Li [37]	p.L237F/ p.P238T	-	AML	70's	Yes
Alkhateeb [9]	p.Gln368	-	MDS	40's	No
Alkhateeb [9]	p.M316Afs	-	MDS-EB2	40's	No

HM; hematological malignancy, MDS; myelodysplastic syndrome, AML; acute myeloid leukemia, CML; chronic myeloid leukemia, NHL; non-Hodgkin lymphoma, MM; multiple myeloma, AA; aplastic anemia, EB; excess blast, RCMD; refractory cytopenia with multilineage dysplasia, MPN; myeloproliferative neoplasm.