Advanced Clinical Research Center

Division of Hematology and Tumor Biology
血液・腫瘍生物学分野

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Advances in next-generation sequencing have expanded our understanding of cancer-associated mutations, but many biological consequences of these mutations, as well as extrinsic factors remain unclear. Our lab focuses on molecular mechanisms underlying hematological malignancies, integrating cell-intrinsic alterations driven by genetic abnormalities with the roles of extrinsic factors such as the microenvironment, aging, and inflammation. Using patient samples and disease mouse models, we integrate molecular biology techniques with data science approaches to investigate unexplored cancer biology.

1. Mechanistic Insights into Biallelic and Monoallelic *DDX41* Mutations in Myeloid Neoplasm

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DDX41 is a newly identified leukemia predisposition gene encoding an RNA helicase, whose germline mutations are tightly associated with late-onset myeloid malignancies. Importantly, germline *DDX41* mutations were also found in as many as ~7 % of sporadic cases of high-risk MDS, conferring the largest germline risk for myeloid malignancies. In typical cases, a germline loss-of-function allele is compounded by a somatic missense mutation affecting the helicase domain in the remaining allele (p.R525H). However, the molecular mechanism by which *DDX41* mutations lead to myeloid neoplasms have not fully been elucidated.

To clarify the role of these distinct *DDX41* alleles, we generated mice models carrying either or both of conditional/constitutive *Ddx41* knock-out (KO) and conditional R525H knock-in alleles. Next, by crossing these mice and further breeding with *Rosa26-CreERT2* transgenic mice, we engineered mice that were wild-type for *Ddx41* ($Ddx41^{+/+}$), heterozygous *Ddx41* KO ($Ddx41^{+/-}$), homozygous *Ddx41* KO ($Ddx41^{-/-}$), heterozygous for the *Ddx41* R525H mutation ($Ddx41^{R525H/+}$), or hemizygous for the *Ddx41* R525H mutation ($Ddx41^{R525H/-}$), in which expression of the mutant allele was induced by tamoxifen administration.

In noncompetitive BM transplantation, most of the recipient mice that were transplanted with BM from $Ddx41^{-/-}$ or $Ddx41^{R525H/-}$ mice died within a month after *CreERT2* induction due to severe BM failure, which was not observed in mice transplanted with

BM from *Ddx41*$^{+/+}$, *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ mice. Transcriptome analysis revealed that stem cells (Kit^{+}Sca-1^{-}Lin^{low} cells) derived from *Ddx41*$^{R525H/-}$ BM-transplanted mice exhibited a significant upregulation of ribosomal genes and several snoRNA genes compared with those derived from *Ddx41*$^{+/+}$ BM-transplanted mice, which could result in abnormal ribosome biogenesis. In addition, *Ddx41*$^{R525H/-}$-derived Gr1+CD11b+ myeloid cells showed a significant upregulation of genes involved in cGAS-STING signaling pathways compared with *Ddx41*$^{+/+}$-derived myeloid cells. However, the survival and cytopenia and the ribosome biogenesis in the stem cells in *Ddx41*$^{R525H/-}$ BM-transplanted mice were improved only marginal when the intact *Sting* alleles were deleted. These results suggest that the impact of the cGAS-Sting signaling on these phenotypes was, if ever, very small, highlighting the role of Sting-independent mechanisms.

In long-term observations, mice transplanted with *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ BM exhibited significantly lower white blood cell counts and anemia, both in primary and subsequent transplantations, although they did not exhibit significant transcriptional changes relative to the wild-type control animals until just before the onset of disease. Some of these mice developed MDS-like phenotypes, including ineffective hematopoiesis and erythroid dysplasia. Stem cells from these mice showed abnormal ribosome biogenesis and reduced expression of interferon response genes. It may be that clones that adapted to the inflammatory environment were gradually selected and contribute to their clonal advantage.

Given that the MDS clones with the *DDX41* R525H somatic allele are commonly observed as a small subclone in patients, we next co-transplanted *Ddx41*$^{+/+}$- and *Ddx41*$^{525H/-}$-derived BM cells with *Ddx41*$^{+/+}$- or *Ddx41*$^{+/-}$-derived BM cells at the ratio of 1:9. The recipient mice showed significantly reduced WBC counts when *Ddx41*$^{+/+}$- or *Ddx41*$^{+/-}$ were co-transplanted with *Ddx41*$^{525H/-}$ -derived BM, suggesting that *Ddx41*$^{525H/-}$-derived hematopoietic cells have negative effect on normal hematopoiesis.

In order to assess the crosstalk between biallelic *Ddx41* mutant cells and their microenvironment cells harboring monoallelic *Ddx41* mutations, we co-transplanted *Ddx41*$^{+/+}$- or *Ddx41*$^{525H/-}$-derived BM cells with *Ddx41*$^{+/-}$-derived BM cells into the constitutive heterozygous *Ddx41* KO mice. The phenotypic and molecular characteristics of these mice are under investigation.

In summary, conditionally introduced compound loss-of function and R525 alleles caused severe BM failure, whereas heterozygous *Ddx41* loss-of function and R525H knock-in alleles are compatible with hematopoiesis, although associated with impaired hematopoiesis and the development of MDS with aging, where an attenuated inflammatory response and ribosome functions may play important roles.

2. Transcriptional and Splicing Dysregulation by *U2AF1* S34F Mutation Contributes to Inflammatory and Migratory Alterations in Hematopoiesis in MDS

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Recent genomic analyses have revealed that mutations in key components of the RNA splicing machinery—including *SF3B1, SRSF2, U2AF1*, and *ZRSR2*—are among the most frequent mutations in patients with myelodysplastic syndromes (MDS). Among these, *U2AF1* mutations are enriched in MDS without ring sideroblasts and in AML with myelodysplasia-related changes, and are associated with poor clinical outcomes. *U2AF1* mutations primarily affect two conserved residues, S34 and Q157, located within the N- and C-terminal zinc finger motifs. Mechanistically, *U2AF1* mutant proteins preferentially recognize the 'UAG' motif upstream of the 3' splice site, causing exon skipping and aberrant 3' splice site selection. Among the reported targets of *U2AF1* mutations are *BCOR* and *GNAS*, both of which are known drivers of MDS, as well as *IRAK4*, a key mediator of innate immune signaling. However, how *U2AF1* mutations affect hematopoietic stem cell (HSC) function and lineage differentiation, and how they shape inflammatory responses or cell–cell interactions, remains incompletely understood.

To address these issues, we generated a conditional knock-in mouse model expressing the *U2af1* S34F allele under the control of *Vav1-Cre* promoters. *U2af1*

mutant mice exhibited macrocytic anemia, leukopenia, and morphological abnormalities, as well as myeloid-skewed hematopoiesis. Transplantation experiments revealed defective HSC reconstitution under competitive conditions.

Bulk RNA-seq of KSL ($Kit^+Sca\text{-}1^+Lin^-$) cells revealed splicing abnormalities in genes associated with myeloid malignancies, including *Hnrnpa2b1*, *Csf3r* and *Gnas*, many of which overlapped with findings from the *Srsf2* P95H mutant mouse model (Kon et al., Blood 2018). In contrast, *U2af1* mutant KSL cells showed specific exon skipping in genes related to inflammatory signaling and RNA metabolism. Gene set enrichment analysis revealed that both *U2af1* and *Srsf2* mutations upregulated pathways related to DNA repair, cell cycle regulation, and RNA processing. However, genes related to cell migration and inflammation were downregulated uniquely in *U2af1* mutant KSL cells, highlighting the mutation-specific nature of splicing factor-driven disease mechanisms in MDS.

To investigate the cell type–specific impact of the *U2af1* S34F mutation, we performed single-cell RNA sequencing (scRNA-seq) on HSPCs and analyzed transcriptional changes across differentiation lineages. In monocyte–dendritic progenitors (MDPs), genes involved in cell migration and motility, as well as inflammatory response pathways, were significantly downregulated. Additionally, adhesion-related genes, including *Cd34* and *Tgfb1*, were markedly reduced, suggesting diminished mobilization capacity and altered interactions with the BM microenvironment. Similarly, in megakaryocyte–erythroid progenitors (MEPs), adhesion and migration-related genes, including *Spn* and *Itga4*, were downregulated, while tumor-related pathways were activated. In contrast, multipotent lymphoid progenitors (MLPs) exhibited upregulation of inflammatory genes. These findings suggest that lymphoid lineage cells, such as MLPs, contribute to the activation of immune responses and inflammatory pathways, while myeloid lineage progenitors, including MDPs and MEPs, exhibit impaired migration and adhesion functions, as well as reduced immune response capabilities, contributing to MDS pathogenesis.

Based on scRNA-seq data, we analyzed known ligand-receptor interactions between cells and predicted that interactions related to cell migration pathways, such as *Cd34–Selp*, *Sell–Cd34*, and *Icam1–Spn*, are altered. Notably, most differentially expressed genes associated with motility and inflammation did not exhibit splicing abnormalities, suggesting these alterations are driven by transcriptional dysregulation rather than splicing defects. Importantly, these transcriptional changes were also observed in bulk RNA-seq data from *U2AF1*-mutant samples from human MDS patients. Similar expression changes were found in CRISPR-engineered MOLM13 cells harboring the *U2AF1* S34F mutation.

In conclusion, our findings demonstrate that the *U2AF1* mutation disrupts hematopoiesis through lineage-specific transcriptional reprogramming, particularly impairing inflammatory and migratory pathways. These results highlight the mechanistic divergence among splicing factor mutations and underscore the therapeutic potential of targeting dysregulated inflammation and cell migration in MDS.

Publication

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