Advanced Clinical Research Center

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

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Cancer arises from genetically diverse cells due to repeated clonal selection of driver mutations that confer survival advantages. Advances in next-generation sequencing have expanded our understanding of cancer-associated mutations, but many biological mechanisms remain unclear. Our lab focuses on uncovering unknown genetic abnormalities and molecular mechanisms underlying hematological malignancies. Using patient samples and disease mouse models, we integrate molecular biology techniques with data science approaches to investigate unexplored cancer biology.

1. Functional analysis of germline and somatic *DDX41* Mutations in the pathogenesis of myeloid malignancies

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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 ~8 % of sporadic cases with 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 mechanisms by which *DDX41* mutations lead to myeloid neoplasms have not been fully 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 (KI) alleles. 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.

First, we assessed cell intrinsic effects of these *Ddx41* alleles, using noncompetitive transplantation experiments. Shortly after tamoxifen administration, 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 (BMF), which was not observed in mice transplanted with BM from *Ddx41*$^{+/+}$, *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ mice. By contrast, the mice transplanted with *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ BM showed significantly reduced WBC counts and anemia in long-term observation in both primary and serial transplantations. Some of the *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ BM-transplanted mice exhibited MDS-like phenotypes, showing ineffective hematopoiesis with evidence of erythroid dysplasia.

Transcriptome analysis revealed that stem cells derived from *Ddx41*$^{R525H/-}$ BM-transplanted mice exhibited a significant upregulation of genes involved in innate immunity, including interferon stimulated genes, compared with stem cells derived from *Ddx41*$^{+/+}$ BM-transplanted mice. In addition, ribosomal genes were significantly deregulated in stem cells from *Ddx41*$^{-/-}$ and *Ddx41*$^{R525H/-}$ BM-transplanted mice, which could result in abnormal ribosome biogenesis and protein synthesis in *Ddx41* mutant cells.

Our results revealed that monoallelic *Ddx41* loss-of function led to age-dependent impaired hematopoiesis, 1while biallelic loss-of function and R525 alleles showed a compromised function of hematopoiesis, where activated innate immunity and impaired ribosome functions may play important roles.

Publication

1. 昆 彩奈, 骨髄異形成症候群における動物モデルを用いた病態解明 (Recent advances in experimental animal models of myelodysplastic syndromes) 月刊血液内科(科学評論社), 2024;89(1):8-13. 2024年7月発行