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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Pathogenic Mechanisms of *DDX41* Mutations in the Development 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 ~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 (most commonly p.A500fs or p.D140fs, depending on the ethnicity) 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 (KI) alleles. *Vav1-Cre* mediated homozygous deletion of *Ddx41* resulted in embryonic lethality, suggesting that Ddx41 is indispensable for normal hematopoiesis. 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.

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 (Kit $^{+}$Sca-1 $^{-}$Lin ^{low} 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, snoRNA and 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.

We also assessed the reconstitution capacity of whole BM cells from different *Ddx41* mutant mice in competitive transplantation experiments. The donor chimerism of $Ddx41^{-/-}$ and $Ddx41^{R525H/-}$ BM-transplanted mice in PB was markedly reduced compared to $Ddx41^{+/+}$ BM-transplanted mice. In contrast, $Ddx41^{+/-}$ and $Ddx41^{R525H/+}$ BM-transplanted micedid not show significant changes in competitive bone marrow reconstitution compared to $Ddx41^{+/+}$ BM-transplanted mice. Given that the MDS clones bearing *DDX41* R525H somatic alleles are typically 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. 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 summary, compound biallelic loss-of function and R525 alleles led to severe BM failure. Monoallelic *Ddx41* loss-of function and R525H knock-in alleles, by contrast, are compatible with hematopoiesis but associated with an impairment of hematopoiesis and the development of MDS with ageing, where activated innate immunity, impaired RNA metabolism and ribosome functions may play important roles.

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