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学位の種類	博士（ 医学 ）
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審査研究科	人間総合科学研究科
学位論文題目	Protective role of residual *Tet2/Tet3* alleles in development of myeloid leukemia（残存 *Tet2/Tet3* アレルが骨髄性白血病発症阻止に果たす役割）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, the author describes the role of Tet2/Tet3, the family members of ten-eleven translocation (TET) methylcytosine dioxygenases, in the development of myeloid leukemia. Loss-of-function mutations in *TET2* are associated with acute myeloid leukemia (AML) and preleukemic hematopoietic stem cells (HSCs) of age-related clonal hematopoiesis, and an age-related decline in the level of *TET3* has been reported in HSCs. Therefore, the author investigated the impact of gradual decrease of TET function by generating and analyzing the leukemia phenotype in an allelic series of *Tet2/Tet3* deficiency in mice. The author found that three-allele disrupted mice required additional secondary mutations to progress to leukemia, whereas four-allele disrupted mice spontaneously developed leukemia within a short period. Her thesis work suggests that the residual Tet2/Tet3 allele plays an important role as a gatekeeper to prevent leukemia progression.
The content is summarized as follows:

（目的　Purpose）

The purpose of this research is to explore the molecular mechanism of the development of myeloid leukemia in mice with *Tet* deficient condition.

（対象と方法　Materials and Methods）

An allelic series of mice with *Tet2*/*Tet3* deficiency were generated by crossing *Tet3*-floxed ($Tet3^{f/f}$) with

Tet2-floxed (*Tet2*$^{f/f}$) mice expressing *Cre* recombinase under the control of the type I interferon-inducible *Mx1* promoter (*Mx-Cre*), and *Tet2*$^{f/wt}$*Tet3*$^{f/f}$*Mx-Cre*$^{+}$ (T2ΔT3), *Tet2*$^{f/f}$*Tet3*$^{f/wt}$*Mx-Cre*$^{+}$ (ΔT2T3), and *Tet2*$^{f/f}$*Tet3*$^{f/f}$*Mx-Cre*$^{+}$ (ΔT2ΔT3) mice were obtained. Bone marrow (BM) cells were established, and gene deletion was confirmed, and used for transplantation experiments. Genomic DNA was extracted from BM and the tail of the mutant mice and subjected to whole exome sequencing to validate somatic mutations. Surface expressions of various lineage markers were examined in BM cells by FACS analysis and qPCR, and histopathologic examinations were performed using peripheral blood cells, BM cells, and spleen.

（結果　Results）

All *ΔT2ΔT3* mice died of aggressive AML at a median survival of 10.7 weeks. In contrast, three-allele deficient mice, *T2ΔT3* and *ΔT2T3*, developed AML with longer latencies, with a median survival of ~27 weeks. Despite the difference in latency, the majority of the *T2ΔT3* and *ΔT2T3* mice developed AML similar to those seen in *ΔT2ΔT3* mice. The penetrance of AML was confirmed by transplantation of BM cells of leukemic *T2ΔT3* and *ΔT2T3* mice to irradiated congenic mice. These BM cells showed c-Kit^{+} cells partially positive for CD16/32 and CD34 that were similar to the primary leukemic BM cells. After treatment with decitabine, a hypomethylating agent, cell growth was dependently inhibited in all AML cell lines.

Quantitative PCR of leukemic BM cells of *T2ΔT3* and *ΔT2ΔT3* mice demonstrated very low levels of *Tet2* expression and some of *ΔT2T3* showed low levels of *Tet3* expression similar to those of *ΔT2ΔT3.* All nine *T2ΔT3* and eight *ΔT2T3* mice with AML showed the inactivation of the remaining nontargeted *Tet2* or *Tet3* allele, respectively, due to exonic loss in either gene or stop-gain mutations in *Tet3*. Recurrent mutations other than *Tet3* were not noted in any mice by whole-exome sequencing. Spontaneous inactivation of residual *Tet2* or *Tet3* allele is a recurrent genetic event during the development of AML with *Tet* insufficiency.

（考察　Discussion）

The AML phenotype seen in both *Tet2/Tet3* three-allele and four-allele disrupted mice were very similar in terms of cell surface antigen expression, penetrance of the diseases in the transplanted mice, and response to an hypomethylating agent in vitro. However, the latencies were significantly longer in the three-allele deficient mice. This suggests the requirement of additional genetic events for leukemia development in these mice. The author has shown that three-allele disrupted mice required additional secondary somatic mutations to progress to leukemia, whereas four-allele disrupted mice spontaneously developed leukemia within a short period, suggesting that the residual *Tet2/Tet3* allele plays an important role as a gatekeeper that prevents the progression of leukemia.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The applicant findings emphasize that the remaining intact *Tet2* or *Tet3* allele may act as a sentinel to prevent HSCs from AML progression. The applicant also shows that some AML cells may depend on the hypermethylation status induced by severely reduced TET enzymatic activity. The applicant suggests that these conditions could be explored as potential therapeutic targets by hypomethylating agents in the future.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on December 23, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.