論文の内容の要旨

論文題目　Recurrent CDC25C mutations drive malignant transformation in FPD/AML
（家族性血小板異常症の進展に際し頻発する CDC25C 遺伝子変異による腫瘍化機構の解析）

氏名　遠矢　嵩

Familial platelet disorder with predisposition to acute myelogenous leukemia (FPD/AML) is an autosomal dominant disorder and is characterized by inherited mild to moderate thrombocytopenia with or without impaired platelet function and a lifelong risk of development into hematological malignancies. Although inherited *RUNX1* mutation is the cause of thrombocytopenia, secondary mutations may have a role in the development and progression of hematological malignancies in FPD/AML, as only about 40 % of FPD/AML patients develop leukemia with a median age of 33 years. Difficulty in studying FPD/AML pathogenesis exists because it is a rare disorder and only approximately 40 pedigrees with FPD/AML have been described in the world so far. Thus we conducted a nationwide survey in Japan to collect samples and make a diagnosis of patients with familial thrombocytopenia or hematological malignancies and applied a high-throughput sequencing strategy to hematological malignancies in FPD/AML, with the aim of identifying novel recurrent driver mutations. As a result, 57 pedigrees were extracted and 7 pedigrees with germline *RUNX1* mutation were diagnosed as having FPD/AML, in which 8 out of 13 patients had developed hematological malignancies. There was no significant difference in platelet count or other clinical data at diagnosis between FPD/AML patients and non-FPD/AML patients.

To identify additional genetic alterations, we utilized whole exome sequencing in two patients with FPD/AML who had RUNX1_p.Phe303fs mutation and developed myelodysplastic syndrome (MDS, patient 1) or myelofibrosis (patient 2) followed by AML. We confirmed 10 and 8 somatically acquired nonsynonymous mutations in these patients, respectively. Interestingly, both patients had a somatic *CDC25C* mutation. Therefore, the other individuals with FPD/AML were investigated by deep sequencing and subclone strategy to determine the frequency of mutations in *CDC25C*. As a result, 7 out of 13 individuals harbored *CDC25C* mutations (54 %). Surprisingly, 3 mutations were detected in 5 individuals without hematological malignancy. Further sequence analysis in 90 sporadic MDS patients and 53 AML patients identified only one *CDC25C* mutation in a MDS patient among 13 MDS patients with *RUNX1* mutation. Next we estimated clonal evolution during leukemic transformation using variant allele frequency (VAF) of validated mutations. In patient 1, a founder clone with *CDC25C* mutation acquired *COL9A1*, *FAM22G*, and *LPP* mutations (group 1), and subsequent emergence of *GATA2* mutation (group 2) triggers full-blown leukemia, whereas another subclone defined by *CHEK2* and other mutations in 3 genes (group 3) relatively regressed. Similar hierarchical progression was observed in patient 2. Single cell genomic sequencing using genome DNA of 63 bone marrow cells from Subject 20 at the AML phase demonstrated that group 1/2 and group 3 mutations were mutually exclusive and thus validated the hierarchical model.

Among the somatic mutations found in Subject 20 and 21, *GATA2* mutation was also identified in Subject 22. This patient developed AML with multilineage dysplasia, leading to the diagnosis of AML-MRC. Remission induction therapies were partially effective and the blast ratio was reduced from 54 % to 5.6 %, while dysplastic feature persisted. Allogeneic stem cell transplantation was successfully performed from an HLA-matched unrelated donor and complete remission with 100 % donor chimerism was obtained. During the treatment, VAF of *GATA2* mutation decreased almost in parallel with blast percentage while VAF of *CDC25C* mutation hovered at a high ratio before transplantation, suggesting that *GATA2* mutation confers leukemia progression in this patient whereas *CDC25C* mutation is associated with pre-leukemic status.

We next investigated the functional impact of *CDC25C* mutation. CDC25C is a phosphatase that prevents premature mitosis in response to DNA damage at the G2/M checkpoint, while it is constitutively phosphorylated at Ser216 throughout the interphase by c-TAK1. When phosphorylated at Ser216, CDC25C binds to 14-3-3 protein, which sequestrates CDC25C in the cytoplasm and inactivates it. Binding capacity with c-TAK1 of mutant CDC25C was reduced compared with wild-type, resulting in decreased phosphorylation status of CDC25C at Ser216, decreased binding capacity with 14-3-3 protein, and accumulation of mutant CDC25C in the nucleus at the G1/S phase. As a result of these characteristics, CDC25C mutants led to enhanced mitosis entry which was exaggerated by irradiation. These results suggest that *CDC25C* mutation results in disruption of DNA checkpoint machinery.

Next we confirmed that FPD/AML-associated *RUNX1* mutations (p.Phe303fs and p.Arg174X) induce DNA damage through transcriptional suppression of several DNA repair genes, with activation of the G2/M check point mechanism. However, we found that introduction of *CDC25C* mutation resulted in enhancement of mitosis entry in spite of DNA damage induced by *RUNX1* mutations.

In conclusion, FPD/AML-associated malignant transformation is formed by stepwise acquisition of mutations and clonal selection, which is initiated by a *CDC25C* mutation in the pre-leukemic phase and further driven by additional mutations. The identification of *CDC25C* as the gene responsible for the transformation will facilitate the diagnosis and monitoring of individuals with FPD/AML who are at an increased risk of developing life-threatening hematological malignancy.