No.	K24-2120	
Project Title	Exploration of the functional role of FOXP1 in the development of secondary myelofibrosis	
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(Form 2)

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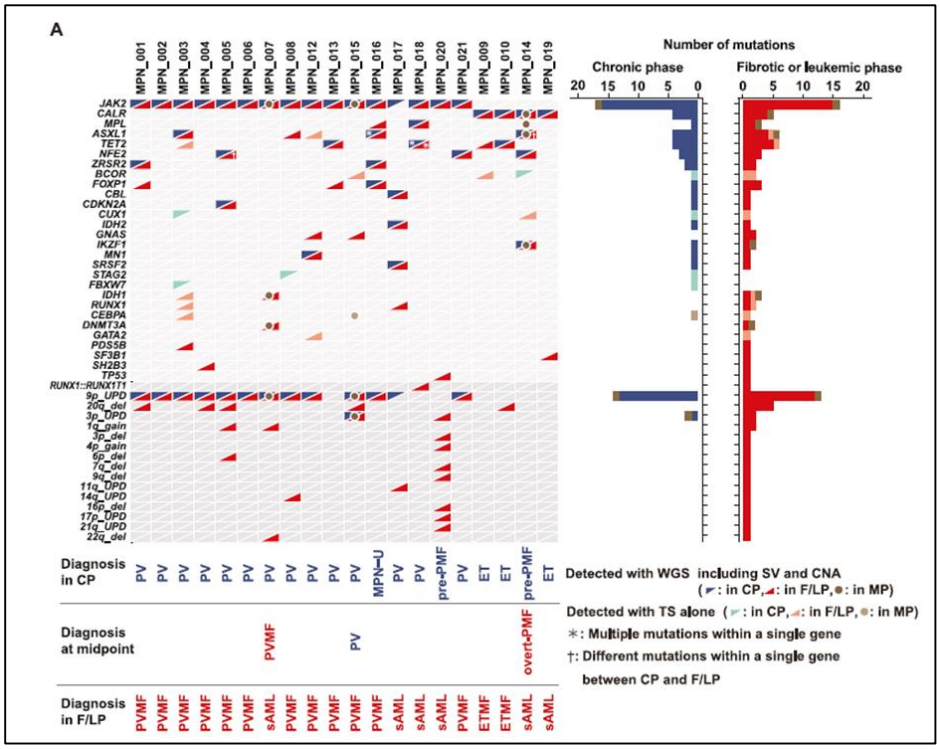
Joint Research Report (Annual/Project Completion)

Project Completion Report

Report

【Background】

Myeloproliferative neoplasms (MPNs) are a type of myeloid malignancy that are characterized by the expansion of a multipotent hematopoietic progenitor stem cell. Philadelphia-negative MPNs in chronic phase have a risk of transformation to secondary myelofibrosis (sMF) and/or secondary acute myeloid leukemia (sAML). Myelofibrosis transformation was observed in 6-14% of patients with polycythemia vera (PV) and 4-11% of patients with essential thrombocythemia (ET) at 15 years, respectively. The prognosis of patients with the transformation of MPNs is dismal. During the last few decades, several genomic studies have revealed the pathogenesis underlying the transformation of chronic phase MPNs and established the classification and prognostic model of patients with MPNs. Myeloid driver mutations other than mutations affecting MPN driver genes (*JAK2, MPL, or CALR*), including *TET2, DNMT3A, ASXL1,* and *EZH2* were identified in 55% of patients with MPNs. Mutations in epigenetic regulators, splicing factors, and RAS signaling have been reported to facilitate myelofibrosis or leukemia transformation. Whole-genome sequencing (WGS) has the potential to reveal the accurate mutational signature and clonal architecture. WGS can also identify novel candidate mutations associated with pathogenesis. Therefore, we performed WGS on serial specimens collected from 20 patients with MPNs in both chronic and advanced phases to explore the underlying mechanism of disease progression.



【Annual report in 2025】

In year 2024, we reported a comprehensive analysis of the genetic mechanisms driving the transformation of Myeloproliferative Neoplasms (MPN) into secondary Myelofibrosis (sMF) and secondary Acute Myeloid Leukemia (sAML). By utilizing Whole Genome Sequencing (WGS) and cohort comparisons, the study identifies specific mutational profiles and patterns of clonal evolution that distinguish different phases of disease progression.

The Role of FOXP1 Mutations

A significant discovery of this study is the identification of novel FOXP1 mutations, which appear to be a specific marker for secondary Myelofibrosis (sMF). These mutations were found in 16.3% of sMF patients, a significantly higher frequency than in overt primary myelofibrosis (2.3%).

Key characteristics of *FOXP1* mutations include:

1) Specificity to Fibrosis: The mutations are primarily protein-truncating (frameshift or nonsense) and are specifically associated with fibrotic transformation.

2) Inhibitor of Leukemic Change: Notably, *FOXP1* mutations were entirely absent in sAML samples. Patients harboring these mutations did not experience leukemic transformation during a median 5.1-year follow-up, suggesting that *FOXP1* may serve as a biomarker for a lower risk of sAML.
3) Clonal Progression: Variant allele frequencies (VAFs) were significantly higher in the sMF phase compared to the chronic phase (CP), indicating clonal expansion during transformation.

Non-Canonical MPL Mutations and Driver Density

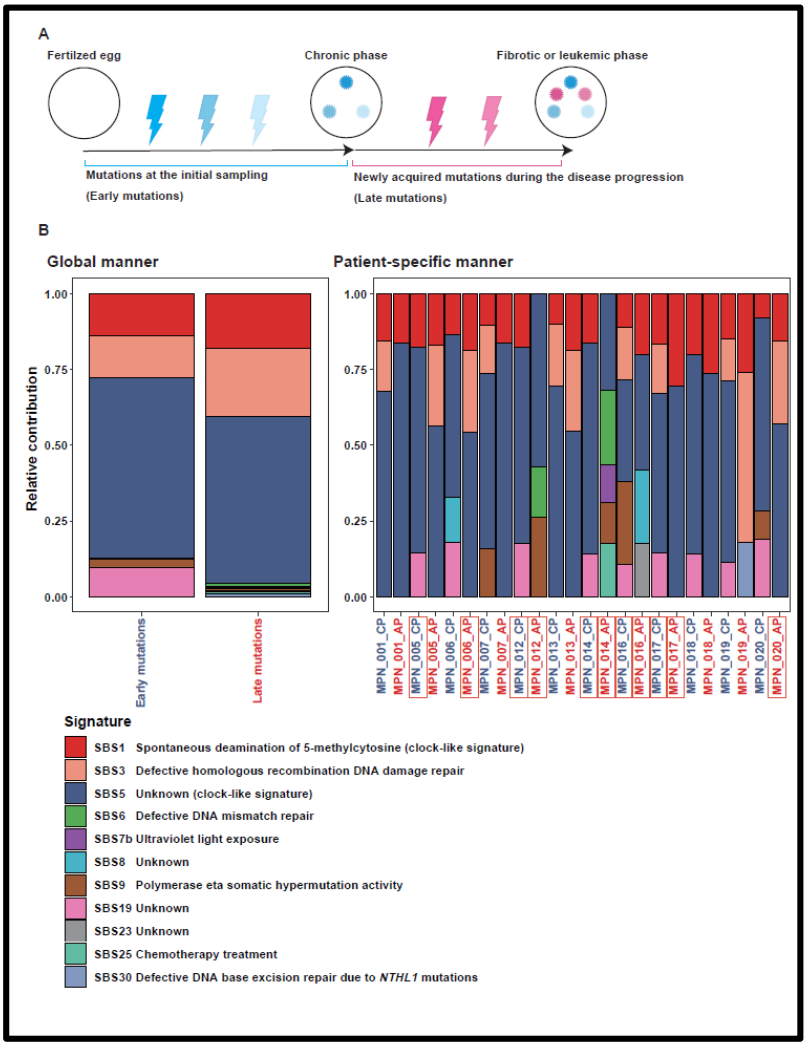
The study also highlights the MPL Y591D mutation. Unlike the common *MPL W515* mutation, *Y591D* often exists as a subclone alongside *JAK2* or *CALR* founder clones. While it is more frequent in MDS and AML than in standard MPNs, its presence in MPN patients suggests it contributes to the transformation process rather than simple myeloproliferative activity. Furthermore, the number of myeloid driver mutations significantly increases during the transition from the chronic phase (1.3 per sample) to the fibrotic/leukemic phase (2.2 per sample).

Clonal Evolution

The researchers categorized the transformation through two lenses:
1) Evolutionary Architecture: Patients exhibited either linear evolution (11 cases) or branched evolution (7 cases).
2) Clonal Dynamics: Most patients (18 out of 20) showed dynamic clonal changes, characterized by the acquisition of new mutations or shifts in clone sizes. Even in "static" cases, expansion of clones without known drivers was observed, hinting at yet-to-be-identified genetic factors.

In 2025, we conducted the following research to further investigate the mechanisms underlying clonal progression in MPN.

Mutational signature profile



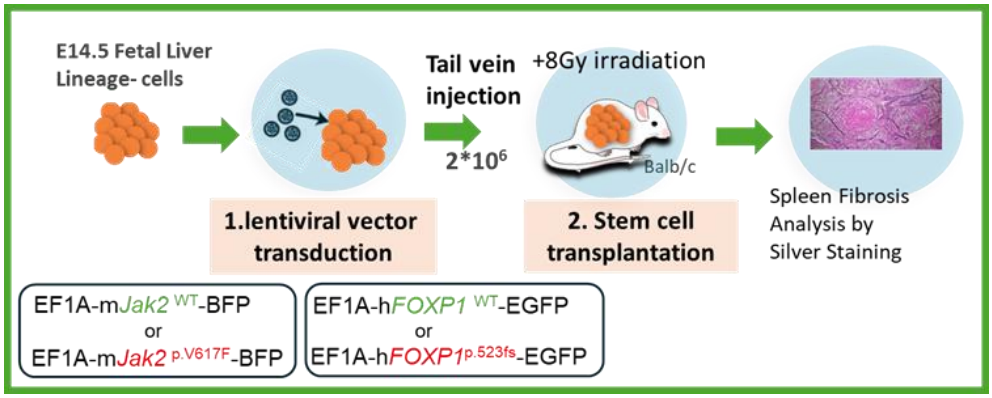
WGS can also reveal accurate mutational signatures and clonal architecture. Furthermore, serial sample collection from individuals could facilitate the elucidation of genetic events specific to the transformation of MPNs. As recent studies using next generation sequencing have focused on specific myeloid driver mutations or exomes, the mutational landscape revealed by WGS during the transformation of MPNs is yet to be fully elucidated. We performed mutational signatures analysis using the Mutational Patterns package in R. Specifically, we first selected the 15 patients who had > 500 SNVs in both CP and AP. Midpoint samples were excluded from this analysis. Next, we classified SNVs of each patient into 'Early mutations' and 'Late mutations' based on the MCF values. Three cases (MPN_002, MPN_015, and MPN_021) had a small number of SNVs (<250) in either of 'Early mutations' and 'Late mutations' to conduct signature analysis and were removed from analysis. We applied maxdelta = 0.02 parameter in MutationalPatterns program to apply only relevant SBS signatures. The signature components from the early and late mutations for each case were merged into global data. We compared the mutational signatures obtained at diagnosis of the chronic phase (early mutations) with those obtained during disease progression (late mutations).-As expected, clock-like signatures represented by SBS1 and SBS5 predominated in both early and late mutations. Of note, SBS19 was specifically observed in the early mutations in 9 of 12 samples examined but not in any of the late mutations. The etiology of SBS19 is not known, but this signature was reported to explain MPN mutations in single cell-based analysis[26].

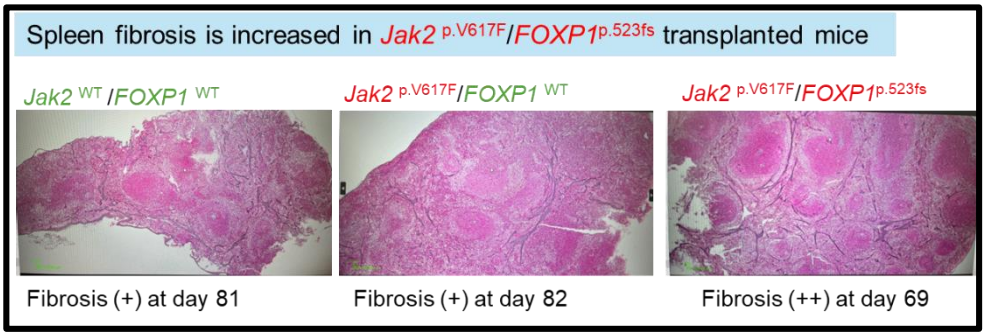
In addition, DNA repair defect-associated signatures (SBS3/SBS6/SBS30) were also seen, with a trend to increase in the late mutations.

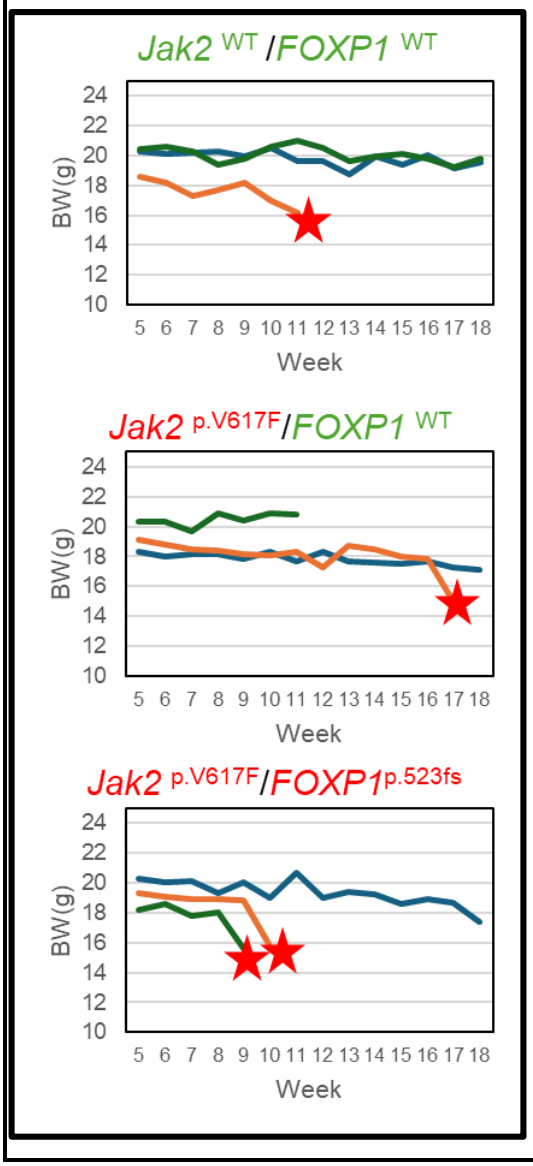
Investigating the Pathophysiological Role of FOXP1 in Myelofibrosis Transformation

To validate our clinical observations regarding the association between ***FOXP1*** mutations and the development of secondary Myelofibrosis (sMF), we established an *in vivo* functional assay using a murine bone marrow transplantation model. The primary objective was to determine whether the loss of *FOXP1* function acts as a synergistic driver alongside canonical MPN mutations to accelerate fibrotic progression.

We utilized a **lentiviral transduction system** to engineer hematopoietic stem and progenitor cells with specific genetic profiles. Two distinct experimental groups were created: a control group expressing only the **JAK2 V617F** mutation—the predominant driver of myeloproliferative neoplasms—and an experimental group co-expressing both the *JAK2* mutation and the newly identified *FOXP1* mutation. These engineered cells were subsequently transplanted into recipient mice that had received a lethal dose of **8 Gy** total body irradiation to ensure successful donor cell engraftment and hematopoietic reconstitution.







Our preliminary results demonstrate a clear phenotypic divergence between the two cohorts. While the *JAK2*-only mice exhibited a standard myeloproliferative phenotype, the mice harboring the **double mutation (JAK2 + FOXP1)** showed a significantly more aggressive disease course. Most notably, histological analysis revealed a marked increase in **splenic fibrosis**, characterized by dense reticulin deposition and disrupted splenic architecture.

This pathological progression was accompanied by severe clinical symptoms. The co-mutated mice experienced **rapid and profound weight loss** compared to their *JAK2*-only counterparts. This wasting syndrome is a hallmark constitutional symptom closely associated with the advanced stages of bone marrow fibrosis and systemic inflammation in human sMF. These initial findings suggest that *FOXP1* mutations are not merely passenger events but play a critical role in driving the fibrotic transformation and increasing the overall symptom burden in the context of *JAK2*-driven MPN.

Conclusions

Through this collaborative study, we were able to elucidate some of the changes in genomic abnormalities associated with the progression of MPN. In particular, since the non-canonical mutation MPLY591 and FOXP1 gene mutations are specifically observed in the progression of MPN, they are attracting attention as mechanisms of clonal progression specific to this disease. Moving forward, we intend to further investigate the mechanisms by which FOXP1 abnormalities contribute to clonal progression and advance research that leads to treatments and preventive measures for disease progression.