PPIL2 is a target of the JAK2-STAT5 pathway and mediates p53 polyubiquitination and degradation

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INTRODUCTION

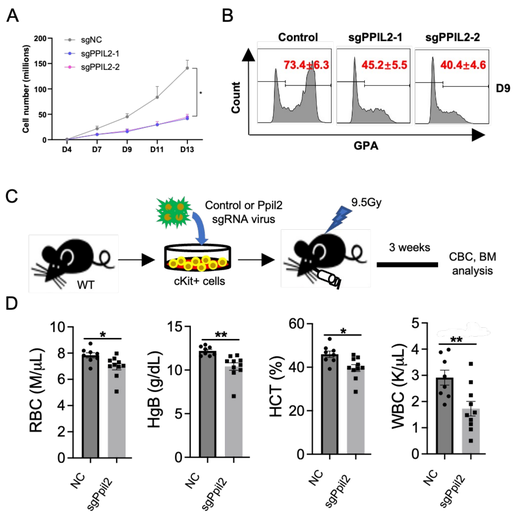
Myeloproliferative neoplasms (MPNs) are a group of bone marrow diseases with excessive production of myeloid cells and increased risk of evolving to acute myeloid leukemia. Majority MPN patients are JAK2^{V617F} mutation [1, 2]. Upregulation of JAK/STAT downstream genes is a typical molecular feature of MPN patients [3]. This indicates the JAK/STAT pathway in the pathogenesis of MPNs and as a valid target for therapy. We previously revealed that Plek2, a downstream target of the JAK2/STAT5 pathway, plays an important role in the pathogenesis of JAK2^{V617F}-induced MPNs [4]. To further explore the mechanisms of the Plek2 signalosome, we performed a comprehensive proteomic analysis of Plek2 interacting proteins and found PPIL2 is a potential novel effector of the signalosome. As a U-box-type E3 ubiquitin ligase, PPIL2 belongs to the cyclophilin protein family, while its biological function has not been clarified [5]. Several studies reported that PPIL2 was involved in cancer metastasis [5, 6]. However, its role in normal and malignant hematopoiesis remains unknown. We sought in this study to investigate the function of PPIL2 in erythropoiesis.

METHOD

- Knockdown PPIL2 by CRISPR-PPIL2 sgRNA in human CD34+ induced erythropoiesis to count cell numbers and perform FACS to test differentiation.
- IP-MS and IP to screening of PPIL2-interacting proteins in erythroid cells.
- Ubiquitination assay were conducted to explore the regulatory mechanism of PPIL2 on TP53.
- Dual-luciferase report assay tests if *PPIL2* transcription is regulated by JAK2/STAT5 pathway.
- Collecting normal and MPNs patient samples and exploring PPIL2 expression levels.
- Transplantation experiment to test the function of Ppil2 in normal and JAK2^{V617F}-induced MPNs.

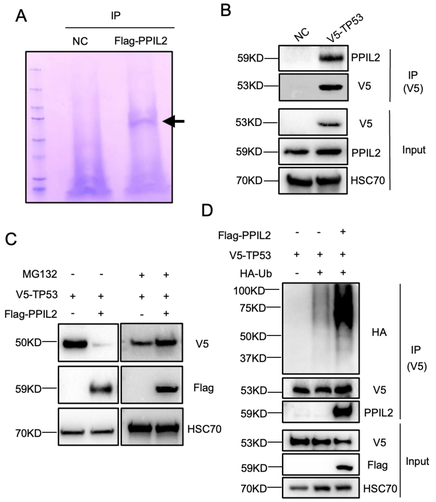
RESULTS

1. PPIL2 positively regulates erythropoiesis



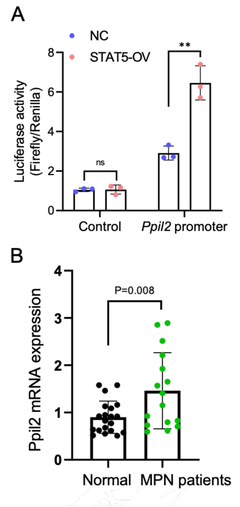
PPIL2 deficiency significantly compromised human and mouse erythroid cells proliferation and differentiation.

2. PPIL2 interacts with TP53 and modulates TP53 stability and ubiquitination



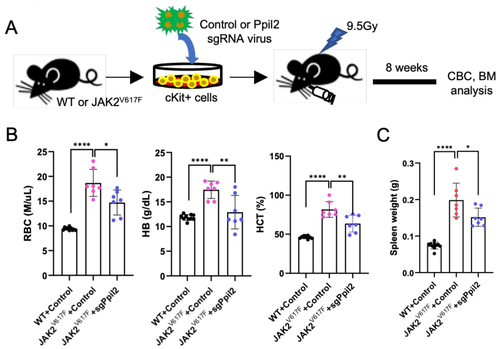
IP-MS assay found PPIL2 interacts with TP53 in erythroid cells (A, B). Downregulation of TP53 by PPIL2 was reversed by MG132 (C). PPIL2 regulates TP53 stability via ubiquitination (D).

3. PPIL2 is a downstream target of the JAK2/STAT5 pathway and upregulated in MPNs



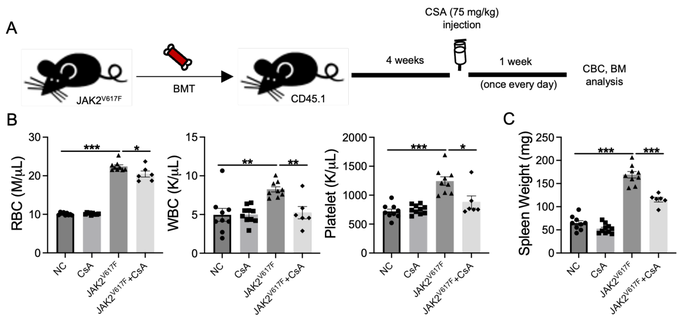
Dual-luciferase report assay showed that STAT5 can bind to *PPIL2* gene promoter (A). PPIL2 is upregulated in MPN samples (B).

4. Knockdown of Ppil2 ameliorates JAK2^{V617F}-induced myeloproliferative phenotypes



PPIL2 deficiency ameliorated JAK2^{V617F}-induced myeloproliferative phenotypes including erythrocytosis, and splenomegaly.

5. CSA treatment ameliorates myeloproliferative phenotypes



PPIL2 inhibitor CsA treatment ameliorated JAK2^{V617F}-induced myeloproliferative phenotypes including erythrocytosis, and splenomegaly.

CONCLUSIONS

1. PPIL2 is required for human and murine erythropoiesis. Knockout of PPIL2 through CRISPR/Cas9 significantly reduced cell proliferation and differentiation.
2. PPIL2 interacts with and catalyzes TP53 polyubiquitination to promote proteosome-mediated TP53 degradation.
3. PPIL2 is a downstream target of the JAK2/STAT5 pathway and is upregulated in a JAK2^{V617F}-positive MPN mouse model and in patients with MPNs.
4. Loss of Ppil2 ameliorated JAK2^{V617F}-induced myeloproliferative phenotypes. The same findings were also observed when treated with PPIL2 inhibitor cyclosporin A.

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