Role of ERK1/2 kinases in megakaryopoiesis, thrombopoiesis, platelet function and thrombosis in myeloproliferative neoplasms

SOHC
SWISS ONCOLOGY & HEMATOLOGY CONGRESS

Experimental Hematology / Oncology

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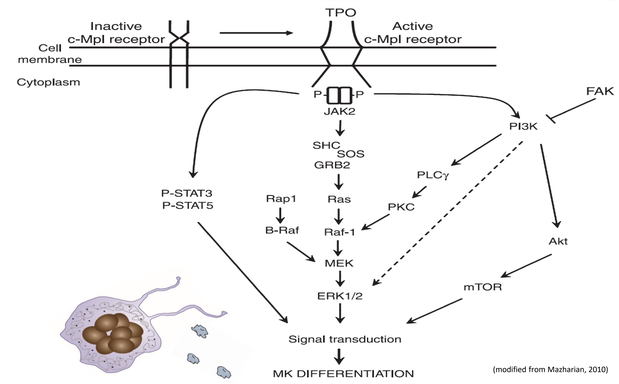
Myeloproliferative neoplasms (MPN) are myeloid malignancies with excessive production of mature myeloid blood cells. They are characterized by constitutively activated JAK2 signaling leading to predominant increase of red cells (polycythemia vera), platelets (essential thrombocythemia) or bone marrow fibrosis (myelofibrosis). Activated JAK2 induces phosphorylation of STAT3/5, the PI3K/AKT pathway and the MAPK pathway including the sequential MEK and ERK kinases, promoting cell proliferation and survival.

Patients with MPN are at risk for thrombo-hemorrhagic events with pooled prevalence of thrombosis of 20% and bleeding of 6.2% at diagnosis. Therefore, the role of megakaryopoiesis and thrombopoiesis as well as characteristics of platelet function in MPN are of high interest. JAK2 and MEK-ERK pathways play important roles for megakaryocyte differentiation, motility and proplatelet formation (*Adam et al, JTH 2008*) and remain activated in MPN despite JAK2 inhibitor therapy (*Brkic et al, Leukemia 2021*). We have previously shown that JAK2 has a regulatory role for megakaryopoiesis and thrombopoiesis (*Meyer et al, Blood 2014*) and it is well established that activated JAK2 signaling in MPN induces increased megakaryopoiesis and thrombopoiesis.

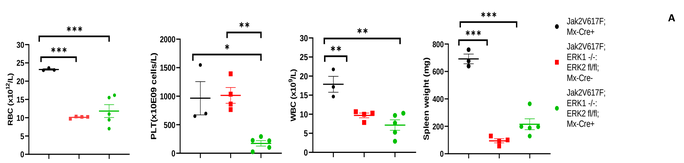
Therefore, we investigate here the roles of ERK1/2 kinases for megakaryopoiesis, thrombopoiesis, platelet function and thrombosis in MPN by genetic targeting.

Aim : Study the role of ERK1/2 in platelet production and function in MPN *in vivo* models to assess the potential of JAK2 and ERK1/2 targeting as a therapeutic approach



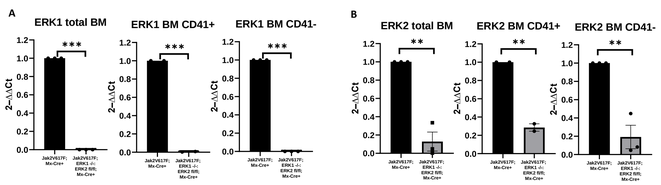
Role of ERK1/2 in platelet production and function in the Jak2V617F primary mouse model

1 ERK deficiency mitigates the MPN phenotype and reduces platelet counts in Jak2 V617F Mx-CRE mice



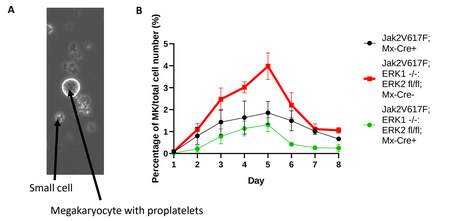
Peripheral blood cell count and spleen weight in primary mice 4 weeks after induction (n=3-5)

2 ERK1/2 deficiency is effectively induced in Jak2 V617F Erk1$^{-/-}$ Erk2$^{f/f}$ Mx-CRE mice



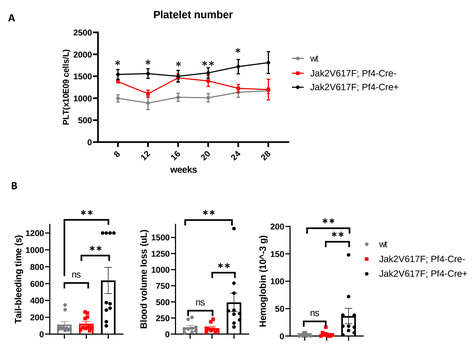
qPCR experiments showing ERK1 (A) and ERK2 (B) mRNA expressions (fold change) 4 weeks after polyI:C induction (n=2-3)

3 ERK1/2 deficiency reduces differentiation capacity of CD41+ cells in Jak2 V617F Mx-CRE mice



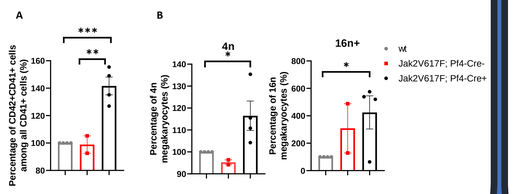
Phase-contrast microscopy image showing a megakaryocyte and small cells (A). Percentage of megakaryocytes on the total number of cells from 1 to 8 days after seeding (B). CD41+ bone marrow cells were collected from primary mice 10 days after induction and cultured with TPO and hirudin. (n=4-8)

4 Jak2 V617F Pf4-CRE mice show thrombocytosis and prolonged tail bleeding



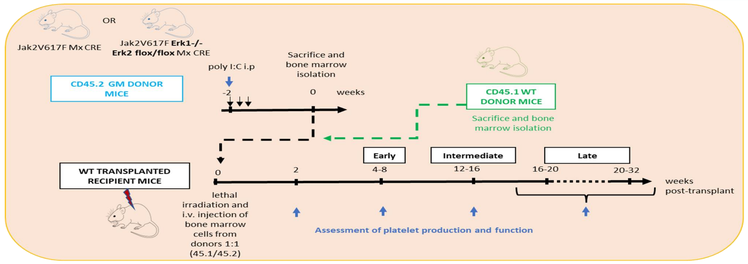
Platelet count over time. *:significant differences between Jak2V617F; Pf4-Cre+ and wt groups (A) (n=5-9). Bleeding time, blood volume loss and total loss of hemoglobin were recorded during tail-bleeding time experiments (B) (n=9-11).Evaluation of Jak2V617F; Pf4-Cre; ERK1-/-; ERK2 fl/fl mice is ongoing.

5 Megakaryocytes show increased differentiation and ploidy in Jak2 V617F Pf4-CRE mice

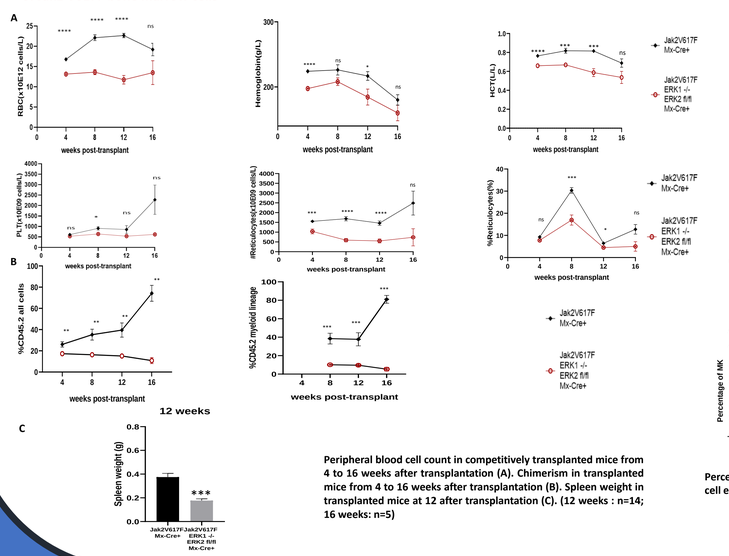


Percentage of CD42c+CD41+ cells in the different mouse lines (A). Ploidy analysis of megakaryocytes from the different mouse lines (B). Experiments were conducted with bone marrow cells after a CD41+ cell enrichment. Cells were stained with CD41-PE, CD42c-FITC, Hoechst and 7AAD. Ploidy and maturation were then assessed by flow cytometry. Data were normalized to wt. (n=2-4)

Role of ERK1/2 in platelet production and function in the Jak2V617F transplanted mouse model

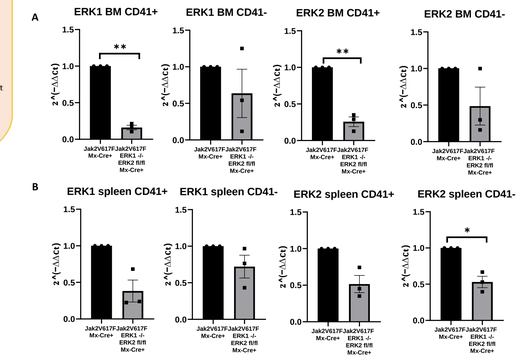


1 ERK1/2 deficiency mitigates the MPN phenotype and reduces mutant allele burden upon competitive transplantation of Jak2 V617F bone marrow cells



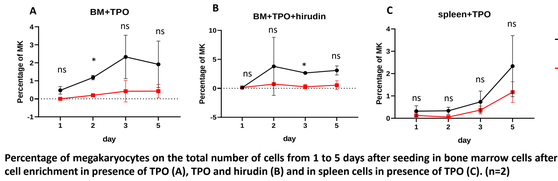
Peripheral blood cell count in competitively transplanted mice from 4 to 16 weeks after transplantation (A). Chimerism in transplanted mice from 4 to 16 weeks after transplantation (B). Spleen weight in transplanted mice at 12 after transplantation (C). (12 weeks : n=14; 16 weeks: n=5)

2 ERK 1/2 expression level is effectively reduced in recipients of Jak2 V617F Erk1$^{-/-}$ Erk2$^{f/f}$ Mx-CRE bone marrow



qPCR experiments showing ERK1 and ERK2 mRNA expressions in term of fold change 12 weeks after competitive transplantation in bone marrow (A) and ERK1 and ERK2 mRNA expressions in term of fold change 12 weeks after transplantation in spleen (B). (n=3)

3 ERK1/2 deficiency impairs megakaryocyte differentiation in Jak2V617F mice



Percentage of megakaryocytes on the total number of cells from 1 to 5 days after seeding in bone marrow cells after CD41+ cell enrichment in presence of TPO (A), TPO and hirudin (B) and in spleen cells in presence of TPO (C). (n=2)

Conclusions

- ERK1/2 deficiency mitigates MPN phenotype and mutant allele burden in a Jak2 V617F Mx-Cre MPN mouse model and impacts on megakaryocyte differentiation
- Further characterization in a Jak2 V617F Pf4-Cre MPN mouse model is ongoing

Presented at SOHC 2024 from 20 – 22 November 2024