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Targeting Endothelial-to-Mesenchymal Transition in a 3D iPSC-Based Model of JAK2V617F+ Myeloproliferative Neoplasms

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INTRODUCTION

Overt myelofibrosis is a late-stage BCR-ABL1 negative myeloproliferative neoplasm (MPN) characterized by progressive bone marrow (BM) fibrosis, causing loss of normal hematopoietic support, alongside inflammatory symptoms, and vascular complications. Alterations of the vascular niche, such as aberrant neoangiogenesis and endothelial-to-mesenchymal transition (EndMT)[1], along with the occurrence of JAK2V617F-mutated endothelial cells (EC)[2,3], significantly impact MPN disease phenotype and progression.

AIM

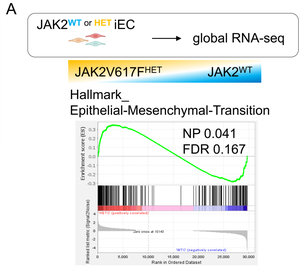
- Explore the impact of JAK2V617F on neoplastic vasculature and its role in MPN-associated fibrosis using patient-specific induced pluripotent stem cells (iPSC)-derived EC (iEC).
- Develop a 3D iPSC-based BM niche model for mechanistic studies of malignant hematopoiesis and drug screening.

METHOD

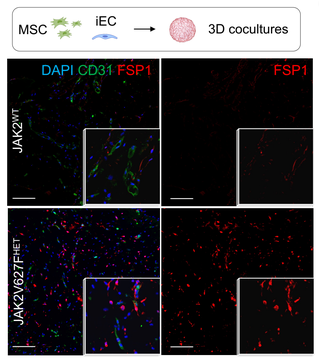
- The iPSC lines used in this study were previously characterized[4], and differentiation into hematopoietic and endothelial lineages was performed as described in prior studies[5,6].
- To model neoplastic vasculogenesis, mesenchymal stem cells (MSC) from healthy donor femoral heads and iEC were suspended in 3D fibrin hydrogels and cultured for up to 5 days.
- To evaluate the anti-EndMT potential of selected drugs, the medium was supplemented with 1000 U/ml IFNα, 250 nM ruxolitinib (RUX), 250 nM nintedanib (NIN), or both ruxolitinib and nintedanib (RUX+NIN, 250 nM each). EndMT was induced using TGFβ (0.5 ng/ml), IL1β (5 ng/ml), and TNFα (10 ng/ml).
- For single-cell RNA sequencing (scRNA-seq), 3D cocultures were generated using MSC, iEC, and iPSC-derived hematopoietic cells (iHC). The iHC included differentiated hematopoietic stem and progenitor cells (iHSPC), megakaryocytes (iMK), monocytes/macrophages (iM), and granulocytes (iGr).

RESULTS

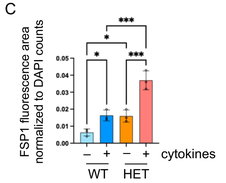
Heterozygously Mutated iEC Exhibit Pro-Mesenchymal Phenotype and EndMT is Alleviated by IFNα and Tyrosine Kinase Inhibitors



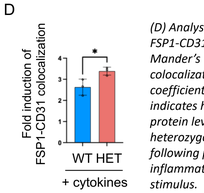
(A) Gene set enrichment analysis reveals a pro-mesenchymal transcriptional signature in heterozygous iEC compared to their WT controls. Abbreviations: NP, nominal p-value; FDR, false discovery rate.



(B) Representative immunofluorescence (IF) images of JAK2^{WT} and JAK2V617F^{HET}-based 3D cocultures, stained for CD31 (green) and fibroblast-specific protein 1 (FSP1; red) to indicate EndMT. Cell nuclei are visualized with DAPI staining (blue). Scale bar, 125 µm

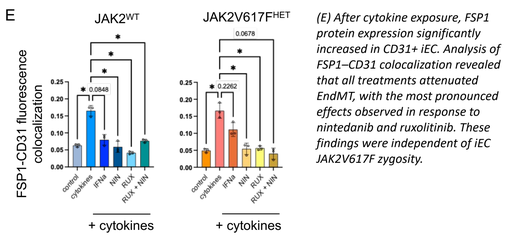


(C) JAK2V617F^{HET} based cocultures show significantly higher FSP1 protein levels compared to WT cocultures, under both stimulated and unstimulated conditions



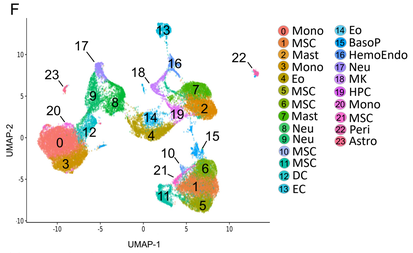
(D) Analysis of duplex FSP1-CD31 IF using Mander's colocalization coefficient (M1) indicates higher FSP1 protein levels in heterozygous iEC following pro-inflammatory stimulus.

- Heterozygous iEC exhibited a more pronounced EndMT phenotype than WT controls in 3D MSC-iEC cocultures, consistent with global RNAseq findings.

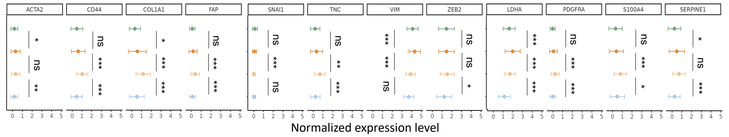


(E) After cytokine exposure, FSP1 protein expression significantly increased in CD31+ iEC. Analysis of FSP1–CD31 colocalization revealed that all treatments attenuated EndMT, with the most pronounced effects observed in response to nintedanib and ruxolitinib. These findings were independent of iEC JAK2V617F zygosity.

IFNα Hampers EndMT Progression in a 3D iPSC-Based BM Niche Model



(F) UMAP plot showing the isolated hematopoietic and stromal clusters. Abbreviations: Mono, monocytes; MSC, mesenchymal stem cells; Mast, mast cells; Eo, eosinophils; Neu, neutrophils; DC, dendritic cells; EC, endothelial cells; BasoP, basophils progenitors; HemoEndo, hemogenic endothelium; MK, megakaryocytes; HPC, hematopoietic progenitor cells; Peri, pericytes; Astro, astrocytes.



*(G) Dot plots with error bars showing the normalized expression levels of EndMT markers in EC from 3D MSC-iEC-iHC cocultures. The color code represents the different genetic backgrounds and treatment conditions: **blue** JAK2^{WT} iEC-iHC cocultures, **yellow** JAK2V617F^{HET} iEC-JAK2^{WT} iHC cocultures, **orange** JAK2V617F^{HET} iEC-iHC cocultures, and **green** JAK2V617F^{HET} iEC-iHC cocultures treated with IFNα (1000U/ml) for 48h prior to scRNA-seq.*

- In the presence of mutated iHC, JAK2V617F^{HET} iEC displayed upregulation of early to mid-stage EndMT markers and increased glycolytic activity, indicating more advanced EndMT.
- The pro-mesenchymal phenotype was partially reversed by IFNα treatment

CONCLUSIONS

- Heterozygous iEC exhibit transcriptional changes that may significantly affect vascular properties, contributing to the clinical phenotype of JAK2V617F+ MPN.
- Our findings demonstrate the anti-EndMT potential of IFNα, ruxolitinib, and nintedanib.
- The presented 3D model of the human MPN BM niche provides a robust platform for disease modeling and drug screening.
- Targeting the vascular niche holds promise for developing novel MPN therapeutic strategies.

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