Transcriptional differences between JAK2-V617F and wild-type bone marrow cells in patients with myeloproliferative neoplasms


The JAK2−V617F mutation is the most common cause of myeloproliferative neoplasms. Although experiments have revealed that this gain-of-function mutation is associated with myeloid blood cell expansion and increased production of white cells, red cells, and platelets, the transcriptional consequences of the JAK2−V617F mutation in different cellular compartments of the bone marrow have not yet been fully elucidated.

To study the direct effects of JAK2−V617F on bone marrow cells in patients with myeloproliferative neoplasms, we performed joint single-cell RNA sequencing and JAK2 genotyping on CD34+-enriched cells from eight patients with newly diagnosed essential thrombocythemia or polycythemia vera. We found that the JAK2−V617F mutation increases the expression of interferon-response genes (e.g., HLAs) and the leptin receptor in hematopoietic progenitor cells. Furthermore, we sequenced a population of CD34− bone marrow monocytes and found that the JAK2 mutation increased expression of intermediate monocyte genes and the fibrocyte-associated surface protein SLAMF7 in these cells.

The JAK2−V617F mutation is a somatic mutation found in most patients with myeloproliferative neoplasms (MPNs) [1]. The mutation causes constitutive JAK−STAT pathway activation in hematopoietic stem and progenitor cells (HSPCs), leading to overproduction of red blood cells, platelets, and white blood cells and/or bone marrow fibrosis. Previous work characterizing JAK2-mutant mouse models [2,3] and MPN patient samples [4] has revealed that JAK2 V617F increases the fitness of hematopoietic stem cells (HSCs) and promotes megakaryocyte–erythroid differentiation. However, it is unclear what molecular mechanisms connect JAK−STAT pathway activation with the observed changes in differentiation and cell division. Furthermore, it is unclear which cell types within the bone

HIGHLIGHTS

- Joint scRNA-seq and JAK2 genotyping were performed for patients with myeloproliferative neoplasms.
- Interferon response genes are significantly more highly expressed in JAK2-V617F hematopoietic progenitors.
- Monocytes with JAK2−V617F have a pro-inflammatory, intermediate monocyte phenotype.
- JAK2−V617F monocytes express SLAMF7, which is associated with fibrosis in MPNs.

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marrow are directly affected by the mutation and which cells are affected by cell-non-autonomous factors. Previous studies have indicated that JAK2 V617F HSPCs have increased JAK–STAT signaling compared with their wild-type (WT) counterparts in the same MPN patient [5,6]. However, these studies generally focused on the mutation’s effects on HSCs and megakaryocyte–erythrocyte progenitors (MEPs). Here, we used joint single-cell RNA sequencing (scRNA-seq) and JAK2 genotyping of bone marrow from polycythemia vera (PV) and essential thrombocythemia (ET) patients to determine the impact of the JAK2 V617F mutation in an unbiased way on multiple bone marrow cell types. In addition to affecting MEPs, we report that the JAK2 V617F mutation directly affects bone marrow monocytes, changing their surface phenotype and increasing expression of SLAMF7, a marker associated with fibrocyte differentiation.

METHODS

Experimental procedures

Cell isolation, scRNA-seq with specific amplification of JAK2, and pre-processing and cell type identification using marker genes were performed using the same methods previously described [4]. All patients were newly diagnosed and treatment naive at the time of sampling and harbored the JAK2 V617F mutation in their peripheral blood. Bone marrow samples from four healthy donors were also collected and scRNA-seq was performed without JAK2 amplification using the same protocol as used on the MPN patients.

scRNA-seq data analysis

Preprocessing of the scRNA-seq data and identification of cells with mutant or wild-type JAK2 amplicon transcripts was performed as previously published [4]. Differential expression analysis comparing cells with and without the JAK2 V617F mutation was performed in scRNA-seq data using the Wilcoxon rank sum test. All raw p values were combined between patients using Fisher’s method and adjusted for multiple comparisons using the Benjamini–Hochberg method. Gene set enrichment analysis (GSEA) was performed using GSEApy to find enriched KEGG biological processes and ChEA/ENCODE transcription factor target groups.

After integration and clustering of data from all patients as previously published, classical, intermediate, and nonclassical subsets were identified in the monocyte population by Louvain clustering the monocyte population only. We used the expression levels of marker genes (classical: CD14, nonclassical: CD16, intermediate: CD74, CD64, HLA-DRA) [7] to manually assign each cluster to a subtype.

Calling somatic mutations in the scRNA-seq data

In the whole-genome sequencing (WGS) data, we identified somatic mutations that occurred only in the JAK2 V617F cells (220 mutations in ET 1, 398 in ET 2). We then detected these mutations in the scRNA-seq data by polymerase chain reaction (PCR) amplification and sequencing (e.g., for a point mutation in UPF1 in patient ET 1) or by calling mutations in the raw 10x transcriptome reads. To call the mutations in scRNAseq data, we extracted all reads mapping to each position mutated in the WGS data using Pysam [8]. Only reads with unambiguous cellular and molecular barcode sequences were considered. Subsequently, we classified reads as mutant or wild type depending on whether the reads contained the mutant or wild-type allele, respectively, requiring a minimum base quality of 30. Mutations that generated false-positive mutant calls in any of the thirty-six 10x Chromium single-cell RNAseq data sets from bone marrow and peripheral blood samples from healthy individuals were considered unreliable and discarded.

Bone marrow monocyte flow cytometry

Flow cytometry of bone marrow monocytes from three patients (ET 1, 3, and PV 3) and three healthy donors was performed to validate some of the results of the monocyte differential expression analysis. SLAMF7 cell surface staining was done on CD14+ cells, as previously published [9].

RESULTS AND DISCUSSION

We analyzed joint single-cell JAK2 genotyping and scRNA-seq data from CD34-enriched bone marrow samples from four ET and four PV patients (Supplementary Figure E1, online only, available at www.exphem.org). Six of these patients were sequenced in a previous study [4]. Two patients (PV 2 and PV 3) also had TET2 mutations, and one patient (PV 1) had a low-frequency EZH2 mutation detected in peripheral blood with a clinical next-generation sequencing (NGS) assay [10].

Bone marrow cells with the JAK2 V617F mutation were intermixed with the cells with WT transcripts when plotted together (Figure 1A–C), suggesting that the mutation does not disrupt the overall structure of the differentiation hierarchy. However, as we reported previously [4], we found that cells with the JAK2 V617F transcript detected were more likely to be megakaryocyte or erythroid progenitors than those with WT JAK2 transcripts (Figure 1A–C), suggesting that JAK2 V617F induces a megakaryocyte–erythroid fete bias. We also found a substantial population of CD14+ bone marrow cells with the JAK2 mutation that do not express CD34 and therefore likely represent monocytes (Figure 1A–C; Supplementary Figure E1).

We compared the transcriptomic profiles of JAK2 V617F bone marrow cells with those from WT cells to determine how the mutation changes gene expression in individual cells in MPN patients. To increase the power of our differential expression analysis, we used the published single-cell WGS data [4] for ET 1 and ET 2 to identify and detect somatic mutations other than JAK2 V617F that uniquely marked the JAK2-mutant clonal population in each of these patients (Figure 1D; Supplementary Table E1, online only, available at www.exphem.org). By leveraging the WGS data, we were able to increase the number of cells that could be assigned as either JAK2-mutant or JAK2-WT by more than twofold for ET 1 and ET 2.

Using the expanded set of genotyped cells, we identified genes that were differentially expressed between JAK2-mutant and JAK2-WT bone marrow cells in ET and PV patients (Figure 1E,F). Although we found few or no significantly differentially expressed genes in HSCs or granulocyte–macrophage progenitors (GMPs; Supplementary Data, online only, available at www.exphem.org), major histocompatibility complex (MHC) class I antigen presentation genes (e.g., HLAs, B2M) were consistently upregulated in JAK2 V617F MEPs, erythroid progenitors, and monocytes in both ET and PV patients. Interferon signaling through STAT1 has been previously associated with increased MHC I expression [11], suggesting that the observed upregulation of these genes in MPN could be caused by increased...
Figure 1 JAK2 V617F megakaryocyte–erythrocyte progenitors (MEPs) have higher expression of pro-inflammatory and antigen presentation genes. (A) UMAP of single-cell RNA sequencing (scRNA-seq) data from bone marrow from eight myeloproliferative neoplasm (MPN) patients, colored by cell type classifications. (B) JAK2-WT (blue) and JAK2-mutant (red) transcripts detected in single cells in the bone marrow of a patient with MPN. (C) Smoothed JAK2 V617F transcript fraction for all patients combined. (D) Detection of mutations associated with JAK2 V617F in patient ET 1. Additional mutations were called using targeted amplification of loci identified from whole-genome sequencing (e.g., UPF1) and by directly identifying somatic mutations in the scRNA-seq data. (E,F) Volcano plots of differential expression analysis results from comparing cells with mutant transcripts to cells with WT transcripts within the MEP, erythroid progenitor, and CD14+ compartments for essential thrombocythemia (ET) patients (E) and polycythemia vera (PV) patients (F). Ribosomal genes, antigen presentation genes, and proteasomal genes are colored in blue, green, and red, respectively. BH=Benjamini–Hochberg; GMPs=granulocyte–macrophage progenitors; HSCs=hematopoietic stem cells; WT= wild type.
Figure 2 JAK2 V617F induces SLAMF7 expression and an intermediate monocyte phenotype in patients with myeloproliferative neoplasm (MPN). (A) Marker gene expression UMAPs of the bone marrow monocyte compartment measured by single-cell RNA sequencing (scRNA-seq) in eight MPN patients. (B) JAK2-WT (blue) and JAK2-V617F (red) transcripts detected in monocytes. (C) UMAP of scRNA-seq data from monocytes colored by transcriptionally defined monocyte subset classifications. (D) Fraction of CD14+ cells with a JAK2-WT or JAK2-mutant transcript detected by scRNA-seq that are classic, intermediate, or nonclassic monocytes. The monocyte subset definitions and color scheme are the same as in (C). (E) Differential expression analysis of monocytes from all eight MPN patients, comparing cells with at least one mutant transcript detected to cells with a WT transcript detected. Ribosomal genes, antigen presentation genes, and proteasomal genes are colored in blue, green, and red, respectively. (F,G) Flow cytometry and SLAMF7 staining of CD14+ cells from three MPN patients and three healthy controls. (F) Gating scheme used to identify SLAMF7. (G) Proportion of CD14+ cells expressing SLAMF7. BH=Benjamini–Hochberg; ET=essential thrombocythemia; PV=polycythemia vera; VAF=variant allele fraction.
JAK–STAT activity, MHC I presentation of T-cell antigens, including JAK2 V617F itself [12], could induce an adaptive immune response against MPN cells, and PD-L1-mediated immune escape through reduced T-cell activation was previously reported in JAK2-mutant MPNs [13]. Upregulation of these inflammation-associated genes in JAK2-mutant MEPs could also contribute to platelet activation and thromboinflammation [14].

We also observed that JAK2 V617F bone marrow monocytes had increased expression of proinflammatory and interferon response genes in both ET and PV patients (Figure 1E,F). These genes are enriched for IRF and STAT targets (Supplementary Figure E2, online only, available at www.exphem.org), suggesting that their upregulation may be due to direct effects of constitutive JAK2 activation. Finally, in JAK2 V617F MEPs, we also noted increased expression of the leptin receptor (Figure 1E), which has been reported to be a marker for long-term engrafting HSCs in mice [15].

We further characterized the transcriptional phenotype of the bone marrow CD34−CD14+ cluster and found that these cells express monocyte genes (Figure 2A). Using expression of monocyte subtype markers [7], we identified classical, intermediate, and non-classical monocyte subsets in our scRNA-seq data and found that JAK2 V617F monocytes were more likely than WT cells to have an intermediate monocyte phenotype (Figure 2B–D). Furthermore, MPN patients had a higher fraction of intermediate monocytes over-all than healthy controls (Supplementary Figure E3, online only, available at www.exphem.org). Intermediate monocytes have been reported to express high levels of antigen presentation genes, secrete both pro- and anti-inflammatory cytokines, and play a role in many infectious and autoimmune conditions [71]. Previous work revealed that patients with JAK2 V617F myelofibrosis have more intermediate monocytes than healthy donors and exhibit dysregulation of cytokine production [16]. This abnormal phenotype is partially reversed by ruxolitinib treatment, suggesting that JAK–STAT signaling contributes to monocyte dysregulation [16]. Our single-cell data suggest that JAK2 V617F acts directly on monocytes to change their phenotype, as we found that individual cells harboring the mutation are more likely to be intermediate monocytes.

Another way monocytes may contribute to MPN pathogenesis is by differentiating into fibrocytes and contributing to bone marrow fibrosis. In JAK2 V617F myelofibrosis patients, CD14+ monocytes have been previously reported to preferentially differentiate into fibrocytes in vitro and express elevated levels of the cell surface marker SLAMF7 [91]. After combining our monocyte scRNA-seq data from both ET and PV patients, we found that SLAMF7 is significantly more highly expressed in JAK2 V617F monocytes than WT monocytes (Figure 2E), although this difference could be due to the higher intermediate monocyte fraction in JAK2-mutant monocytes.

Flow cytometry revealed that ET and PV patients have a higher fraction of SLAMF7+ bone marrow monocytes than healthy donors (Figure 2F,G), suggesting that SLAMF7+ monocytes may play a pathogenic role even in MPN subtypes not defined by bone marrow fibrosis. Consistent with this, none of the patients in our study had evidence of significant reticulin fibrosis in the bone marrow (four patients had myelofibrosis IMF grade 0, one patient (ET 4) had MF grade 0–1, and three patients (ET 2, PV 1, and PV 3) had MF grade 1; Supplementary Figure E1A). Inhibition of SLAMF7 with the monoclonal antibody drug elotuzumab has been reported to suppress fibrocyte differentiation and prevent progression in vitro and in vivo models of myelofibrosis [91]. Our results also suggest that the presence of JAK2-mutant monocytes could be investigated as an early biomarker of myelofibrosis risk in ET and PV patients.

In summary, we found that the JAK2 V617F mutation increases the expression of STAT signaling targets (e.g., antigen presentation and other proinflammatory genes) in HSPCs as well as monocytes. Our results suggest that the JAK2 mutation could lead to a pathogenic proinflammatory, profibrotic phenotype in bone marrow monocytes, and that this population should be further investigated to determine what role it plays in the clinical manifestations of ET and PV and in progression to myelofibrosis.

Conflict of interest disclosure

A.M. has consulted for Janssen, PharmaEssentia, Constellation and receives research funding from Relay Therapeutics. E.S.W. reports personal fees from Jazz Pharmaceuticals, Takeda Pharmaceutical Company, Novartis, and Pfizer. F.M. is the co-founder of an oncology company. J.S.G. has consulted for AbbVie, Takeda, and Astellas and receives research support from AbbVie, Genentech, Prelude, AstraZeneca, and Eli Lilly. D.J.D. receives research support from Glycomimetics, Novartis, AbbVie, and Blueprint Medicines and has consulted for Incyte, Jazz, Novartis, Pfizer, Shire, Takeda, Amgen, Forty-Seven, Agios, Autolo, and Blueprint Medicines. G.S.H. has received research support from Bayer, Merck, Incyte, and Constellation and has received honoraria from Constellation, Jazz, Novartis, and Celgene/BMS. R.M.S. has advisory board, DSMB, and/or steering committee membership at Syntrix ACI Clinical, Takeda, Elevate Bio, Syndax Pharma, AbbVie, Syros, Gemoab, BerGenBio, Foghomer Thera, GSK, Aprea, Innate, Actinium, and OncoNova. M.R.L. has received research support from AbbVie and Novartis.

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SUPPLEMENTARY MATERIALS

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REFERENCES