



Identification of the key Genes and Molecular Pathways in Essential Thrombocythemia Associated with JAK2V617F Mutation Using Bioinformatics Approach

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Background

Essential thrombocythemia (ET) is a chronic myeloproliferative neoplasm (MPN) characterized by significant heterogeneity in clinical course, laboratory profile, and tendency to develop thrombosis, hemorrhage, and myelofibrotic or leukemic transformation [1]. JAK2V617F mutation is one of the most driver mutations for ET, is found in approximately 55% of patients. Thrombotic complications are the leading cause of extensive morbidity and premature mortality in ET with a prevalence of 20.7% and they are accounted for 35-70% of the total mortality in MPN patients. Thrombotic events were reported more often in JAK2V617F positive patients than in JAK2V617F negative.

Objective

This study aimed to identify the differentially expressed genes (DEGs) and pathways in JAK2V617F positive ET using integrated bioinformatics analyses.

References

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Materials & Methods

Microarray data selection and identification of differentially expressed genes (DEGs)

The microarray expression dataset GSE55976 was selected from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/gds/>) to identify the differentially expressed genes (DEGs) in JAK2F617V ET using comprehensive bioinformatics analysis. The GSE55976 dataset included gene expression profile of mature granulocytes and pluripotent hematopoietic CD34+ stem cells from peripheral blood of ET, PV, MF and CML patients compared to healthy individuals. To identify the DEGs in JAK2F617V ET, the gene expression profiles of 11 samples from CD34+ stem cells (3 JAK2V617F positive ET patients, 3 JAK2V617F negative ET patients and 5 healthy individuals) were selected.

GEO2R tool (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>) was used to screen DEGs in JAK2V617F positive ET patients by comparison with healthy individuals and JAK2V617F negative ET patients. An adjusted p-value <0.05 and |log2FC| >1 were used as the criteria for DEG.

Functional annotation clustering and enrichment analysis of DEGs in JAK2V617F

The Database for Annotation, Visualization, and Integrated Discovery (DAVID; <https://david.abcc.ncifcrf.gov>) online database[5] was used to identify clusters and functional genomic annotations of DEGs, with a cutoff p-value < 0.01. The Gene Ontology (GO) enrichment include (biological process (BP), molecular function (MF), cellular component (CC)) and the kyoto encyclopedia of genes and genomes (KEGG) pathway analysis were performed to predict the potential genomic annotation of DEGs.

Results

A total of 58 DEGs were identified in JAK2V617F positive ET patients compared to healthy controls. Out of the 58 DEGs, 12 genes were common overexpressed in both JAK2V617F positive patients and JAK1V617F negative ET patients, 32 genes were overexpressed in JAK2V617F positive while no differences in this 32 genes expression were found between JAK2V617F negative ET and controls. The remaining 14 genes were overexpressed in JAK2V617F positive ET only . i.e not expressed in JAK2V617F negative ET ((ANKRD13A, ATP6V0E1, HERPUD1, HLA-DRB3, IGK, KIAA0930, LTB, PILRA, PYCARD, RAB8B, SNAP23, SON, VCAN, YWHA

GO analysis revealed that, the 14 genes associated with JAK2V617F were significantly enriched in the components of biological processes (BP), including cell communication, immune response and leukocyte activation. The cellular components (CC) category indicated an enrichment of genes associated with the membrane and cytoplasmic vesicle. The most significant GO terms describing molecular functions (MF) for the enriched genes were protein domain specific binding and receptor binding (Figure 1). Pathway analysis revealed that most of the genes in this dataset were involved in immune system pathways, membrane trafficking pathways and cell adhesion molecules (CAMs).

Network clusters analysis, identified 3 significantly clusters. For Cluster 1 and 2, the genes were significantly enriched in cell membrane and transmembrane region. Cluster 3 was significantly enriched in immunoglobulin-like domain and glycosylation (Table 2). Furthermore, the analysis of PPIs identified the SNAP23 gene as one of the highly connected genes in this dataset

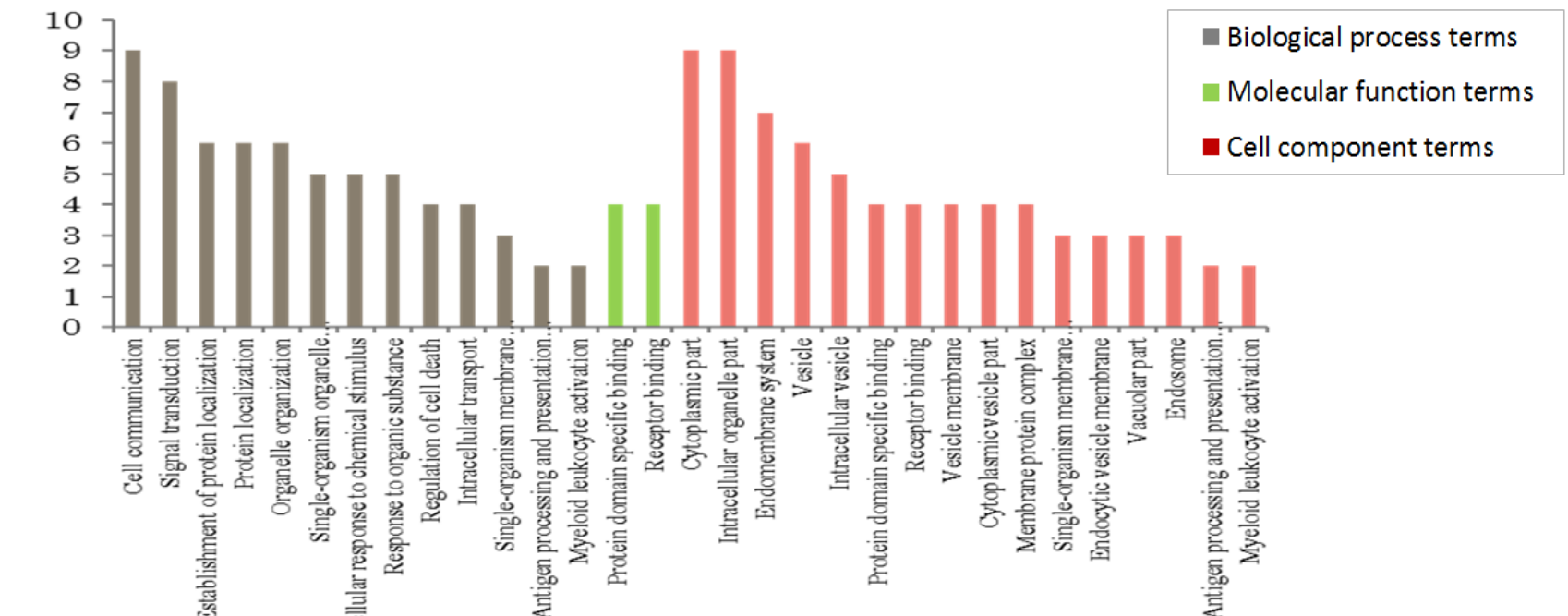


Figure 1 Functional enrichment analysis of overexpressed DEGs in JAK2V617F positive ET

Table 2 Enrichment analysis for the overexpressed DEG clusters in JAK2V617F ET

Cluster	Genes included	Enrichment analysis
Cluster 1	ANKRD13A, ATP6V0E1, HERPUD1, HLA-DRB3, LTB, PILRA, RAB8B, SNAP23.	Cell membrane, membrane, plasma membrane
Cluster 2	ANKRD13A, ATP6V0E1, HERPUD1, HLA-DRB3, LTB, PILRA, PYCARD, RAB8B, SNAP23, VCAN.	Signal transduction, membrane helix, transmembrane region,
Cluster 3	SON, ANKRD13A, HERPUD1, HLA-DRB3, LTB, PILRA, VCAN	Immunoglobulin-like domain, glycosylation,

Discussion

This data manifest that majority of the overexpressed gene in JAK2V617F ET were localized on the cells membrane and involved in cell communication and immune response. The cross talks between inflammation and thrombosis was previously described. More interesting finding in this dataset that SNAP23 was identified as one of the highly connected genes. SNAP23 known to regulates platelet granule secretion. This give insight into SNAP23 as potential therapeutic target in JAK2V617F ET.