Longer Life Foundation – Final Report
Project Title: Functional Dissection of Age-Related Differences in Disease Phenotype in Polycythemia Vera
PI: Stephen Oh, M.D., Ph.D.

Abstract

The overall objective of this study is to utilize phenotypic and functional genomics tools to delineate age-related differences in disease phenotype associated with PV. We hypothesized that intrinsic differences in the composition of genetic changes in the malignant clone dictate these age-related clinical features. We therefore proposed to perform genomic analyses (exome sequencing) on a cohort of ten younger (age ≤ 45) and ten older (age ≥ 65) patients with PV, to delineate the constellation of genetic alterations that define these two subgroups of patients with distinct clinical characteristics. To characterize phenotypic and functional differences between these two subgroups, we proposed to generate and functionally characterize patient-derived induced pluripotent stem cells (iPSCs) via hematopoietic development and self-renewal assays, as well as intracellular signaling analyses via mass cytometry. In addition, we proposed to utilize CRISPR site-directed gene editing to revert *JAK2* V617F and other driver mutations in patient-derived iPSCs from younger and older patients. Taken together, these studies were expected to identify intrinsic genetic, phenotypic, and functional differences responsible for age-associated clinical characteristics in PV. A deeper understanding of the underlying biology that drives disease phenotype would potentially have important ramifications for PV prognostication and development of improved therapeutic approaches.

Lay Summary

Polycythemia vera is a chronic blood cancer that can cause severe complications and early death. Differences in clinical features and outcome have been observed in younger (age ≤ 45) versus older (age ≥ 65) patients with PV. We have sought to understand the basis for these differences, and therefore proposed a series of genetic and functional studies to address these issues. The long term goal of this work is to leverage the findings from this study to improve longevity for patients with PV.

Introduction

Myeloproliferative neoplasms (MPNs) are chronic hematologic malignancies that are clonally derived from hematopoietic stem/progenitor cells (HSPCs) and are typified by overproduction of myeloid lineage blood cells. Polycythemia vera (PV) is a subtype of MPN that is defined by erythrocytosis and associated with thrombotic complications and a propensity for transformation to myelofibrosis (MF) or secondary acute myeloid leukemia (sAML). The *JAK2* V617F mutation, which is present in >95% of patients with PV, activates JAK-STAT signaling and drives hyperproliferation, manifested clinically in increased blood counts.

The estimated prevalence of PV is 50/100,000 persons, with ~150,000 persons living with PV in the United States. Several studies have shown that PV life expectancy is substantially impaired compared with age-matched controls. Common causes of death in PV include thrombotic complications, hemorrhage, and transformation to MF/sAML. PV tends to occur in older patients (median age at diagnosis of 61 years), but a substantial proportion of patients are diagnosed at younger ages. Recent studies have identified age-related differences in disease characteristics and clinical outcomes in PV. Younger patients (age ≤ 45) have lower *JAK2* V617F allele burdens and lower white blood cell counts. Overall rates of thrombosis are similar, but sites of thrombosis vary with age; e.g., splanchnic vein thrombosis occurring more frequently in younger patients. In addition, transformation to MF and sAML, typically associated with advanced age, occurs with similar frequencies in young vs. older patients. The biological explanation for these observations is unclear.

In addition to *JAK2* V617F, recurrent mutations in several other genes (e.g., *TET2, ASXL1, DNTM3A, SF3B1)* have been identified in PV. *TET2* mutations may occur either before or after acquisition of *JAK2* V617F, and recent studies have suggested that the order of mutation may influence disease phenotype. However, the full spectrum of driver mutations in PV, and most importantly their potential segregation with age, has not been comprehensively delineated. Phenotypic and functional studies aimed at understanding age-related differences in PV have also been hampered by the limited availability of primary samples for these studies.

Methods

Genomic DNA from bulk peripheral blood mononuclear cells and matched normal tissue (skin or sorted CD3+ T cells) were isolated from samples obtained from 10 younger (age ≤ 45) and 11 older (age ≥ 65) PV patients. Enhanced exome capture sequencing utilizing the standard IDT exome capture panel plus additional

probes for AML and MPN-specific genes was performed, followed by identification of somatic mutations and comparative analysis between young and old PV samples.

Results

Comparative analysis of somatic mutations was performed on 7 younger and 10 older patient samples (four samples were removed from the analysis due to sample contamination/artifact issues). 103 non-synonymous mutations were identified overall. A significantly higher mutational load was observed in the older group ($p = 0.0025$, Mann-Whitney). All patients were confirmed positive for the *JAK2* V617F mutation, with a trend toward higher *JAK2* mutant allele burden in the older group. The majority of patients were heterozygous (70%) or homozygous (12%) for the *JAK2* 46/1 haplotype, a germline variant associated with *JAK2* V617F-positive MPNs, with no significant differences seen between groups. Putative secondary driver mutations (in addition to *JAK2* V617F) were identified in the majority of older PV patients, whereas in each of the younger PV patients *JAK2* was the only driver mutation present. Inferring from the observed variant allele frequencies it appeared that the majority of older PV patients acquired these cooperating mutations after *JAK2*. This suggests that these mutations are not merely a function of aging but could be playing a role in PV initiation/development.

Discussion

These results suggest that younger and older PV patients exhibit distinct mutational profiles. While the increased number of total mutations observed in older PV patients complies with the notion of acquiring mutations as a function of normal aging, the finding that several secondary mutations were likely acquired after *JAK2* V617F suggests the possibility of a more specific role for these mutations in conferring the distinct clinical characteristics observed in older PV patients. Validation of these findings via single cell-derived colony genotyping is in progress. Functional studies of primary cells from both patient cohorts (including the characterization of patient-derived iPSCs) will be explored in the future. Based on the promising results from this study thus far, submission of an R21 grant application to support these studies is anticipated within the next year.