PATHWAY ANALYSIS IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS AND MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS BY WHOLE EXOME SEQUENCING

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Introduction: The molecular biology of Philadelphia-negative (Ph-negative) myeloproliferative neoplasms and myelodysplastic/myeloproliferative is not completely elucidated. It is known that activation of molecular pathways that lead to increased cellular proliferation (e.g. JAK-STAT pathway) and epigenetic abnormalities (e.g. DNA methylation and histone modification abnormalities) are a recurrent feature of these diseases. Through the use of whole exome sequencing, we can interrogate the genetic landscape of these neoplasms in an unbiased manner, and determine the most commonly mutated set of genes and pathways.
Methods: Paired DNA (sorted CD66b-granulocytes/skin biopsy) from 131 patients with MPNs or MDS/MPD was subjected to whole exome sequencing (N=125; Agilent SureSelect) whole genome sequencing (N=6) on an Illumina HiSeq 2000 platform. Diagnosis included primary myelofibrosis (MF; N=60), essential thrombocythemia (ET; N=39), polycythemia vera (PV; N=12), chronic myelomonocytic leukemia (CMML; N=11), systemic mastocytosis (MS; N=5), MDS/MPD-Unclassified (N=2) and post-MPN AML (N=2). Tumor/normal coverage was 150x/60x (exome) and 70x/30x (genome). Somatic variants calls were generated by combining the output of Somatic Sniper (Washington University), Mutect (Broad Institute) and Pindel (Washington University). The combined output of these 3 tools was further filtered by in-house criteria in order to reduce false-positive calls. Analysis of driver mutations was made with the MutSigCV software and network analysis was done with Cytoscape, using the Reactome FiViz plugin.
Results: We identified a total of 4272 somatic mutations in all patients, including 3868 single nucleotide variants and 404 short insertions/deletions. Using the MutSigCV algorithm, the following genes were identified as the most significantly mutated driver genes in Ph-negative MPNs and MDS/MPDs (in order of significance): *JAK2, CALR, ASXL1, TET2, DNMT3A, SRSF2, SH2B3, CBL, NRAS, U2AF1, ZRSR2 and EZH2.* Novel mutations in genes recurrently mutated in these disorders were found, including *MPL, SH2B3, ASXL1, TET2, DNMT3A, PHF6, CUX1* and *JARID2*. We found mutations in genes previously found to be involved in other neoplasms, such as *ABCA12, ELF1, EP300, CREBBP,* and *IRF1*. Network analysis through the Cytoscape platform revealed that mutations in 222 genes clustered into 12 different protein networks. The major clusters identified included cluster 0 (enriched for mutations in genes in JAK-STAT and Ras pathway), cluster 1 (mutations in genes associated with P53 pathway and DNA damage repair), cluster 2 (PRC2 histone methylation and DNA methylation), cluster 4 (mRNA splicing genes) and cluster 5 (cadherin pathway). Further analysis to better discern the clinical features of patients harboring mutations in each one of these networks is ongoing and will be presented.
Conclusions: Our study represents one of the largest cohorts of patients with MPN and MDS/MPN to be evaluated by whole exome sequencing. We could identify several novel mutations in genes recurrently mutated in these disorders, and identified mutations in genes previously implicated in other neoplasms. Network analysis revealed the importance of novel pathways in the pathogenesis of MPNs. Sequencing of larger cohorts of patients is needed to fully delineate the whole spectrum of genetic abnormalities in these neoplasms.