P997 PROTEOMIC ANALYSIS ON PLATELETS OF ESSENTIAL THROMBOCYTHEMIA PATIENTS UNDERSCORES THE ROLE OF MITOCHONDRIA IN JAK2 V617F PLATELET REACTIVITY AND FUNCTION

**Topic:** Myeloproliferative neoplasms - Biology & Translational Research

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**Background:**

Essential thrombocythemia (ET) is a heterogeneous disease subdivided into five genetic groups according to WHO, based on the common MPN driver mutations (JAK2 V617F, MPL W515K/L, and CALR Type I&II). Current treatment strategies include anti-platelet (e.g. acetylsalicylic acid (ASA)) or cytoreductive agents (e.g. hydrea (HU)) and target mainly complications related to platelet dysfunction (i.e. hemorrhage/thrombosis). Platelet activation is an energy demanding process fueled mainly by a dynamic equilibrium between mitochondria oxidative phosphorylation and glycolysis. Importantly, altering the platelet catabolic response to activation has been shown to prevent thrombus formation. JAK2 V617F platelets have been reported to be more activated than CALR mutated platelets, as well as JAK2 mutated patients have a greater thrombotic risk in comparison to other ET groups. However, the cause of this phenotype is not completely understood.

**Aims:**

In this study we aimed to analyze the proteome of ET platelets and to characterize their functional properties according to the mutational background and treatment regimens.

**Methods:**

22 healthy donors (HD) and 67 ET patients have been included in the study. Most of the patients were treated with low dose aspirin (ASA), anagrelide (ANA) or hydrea (HU) or a combination of these. Specifically, 35 out of the 67 ET platelet samples and 8 out of 22 HD were subjected to label-free quantitation mass spectrometry (LFQ-MS). All HD and ET platelets were also analyzed for surface marker expression, degranulation and aggregation capacity by flow cytometry.

**Results:**

Hierarchical clustering of the mass spectrometry data revealed different proteomic profiles between HD and ET mutational groups and specifically between JAK2 V617F and CALR I and CALR Type II treated and/or untreated platelets. In general, HD platelets were more enriched for proteins related to platelet activation, and degranulation.
as compared to ET platelets and clustered next to JAK2 V617F ASA-treated platelet samples (Figure 1). In particular, the JAK2 V617F ASA-treated platelets presented significant enrichment in platelet activation metabolic and mitochondrial proteins in contrast to the CALR Type I and Type II ET platelets. Of note, no peptides corresponding to the mutant CALR were detected using label-free MS methods. Surface marker expression was variable among ET patients. However, CD49B and CD36 surface markers were the most affected among the JAK2V617F and CALR Type I and II platelets and their levels (mean fluorescence intensity-MFI) were inversely correlated. With regards to platelet aggregation, JAK2 V617F platelets presented similar or lower levels when compared to HD platelets, in line with their proteomic profiles. Differences in aggregation and degranulation levels were also observed between CALR Type I and Type II platelets specifically in the untreated condition.

Summary/Conclusion:

ET platelets are functional but they show different capacities to respond upon various stimuli and treatment regimens. JAK2 V61F platelets are more activated than any other ET group according to their proteome profile, which is not fully reflected by the functional assays. Mitochondrial activity arises as an important factor in the control of platelet reactivity and it could be critical in disease management and treatment strategies, specifically for the JAK2 V617F patients.

References

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