P979 EXOSOMES IN POLYCYTHEMIA VERA: "MINI PLATELETS" WITH THROMBOGENIC POTENTIAL

Topic: 15. Myeloproliferative neoplasms - Biology & Translational Research

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Background:

Polycythemia vera (PV) is characterized by clonal proliferation of myeloid progenitor cells that is caused by activating mutation in the Janus Kinase (JAK)2 gene. Patients with PV tend to develop thrombotic complications across the vasculature tree. Yet, how PV-clonal cells induce pro-thrombotic environment is still under investigation. Secreted by all types of cells, exosomes are nano-scaled particles which carry a molecular cargo that reflects their cell of origin. Exosomes travel in blood, may reach distant sites and affect various physiological and pathological processes. These features led us to think that PV-derived exosomes (hereafter PV-exosomes) carry the mutated JAK2 oncogene and contribute to the thrombotic manifestations seen in PV.

Aims:

1. Determine whether PV-exosomes carry mutated JAK2 transcripts.
2. Determine the prothrombotic potential of PV-exosomes and their effect on endothelium.

Methods:

Exosomes were isolated from eythroleukemia (HEL 92.1.7) cell line (positive for mutated JAK2) and from patients with PV by serial ultracentrifugation. To confirm the presence of exosomes, electron microscopy images were obtained and to quantify their concentration nanoscale tracking analysis was applied (Malvern).

The exosomes were stained with the FM-4-11 lipophilic dye and their uptake by HUVECs and HaCaT cells was followed by flow cytometry. To determine the presence of JAK2 transcript, a 465 bp long DNA segment within exon14 of JAK2 was amplified by qRT-PCR and by Sanger sequencing JAK2 mutational status was determined. Thrombin generation assay (TGA) (Ceveron®, Alpha, Technoclone) was used to assess the thrombotic potential of PV-exosomes. Platelet activation status was assessed by the expression of PAC-1, CD-62 and CD-63 in platelets and Trans Epithelial Electric Resistance (TEER) (Electric cell substrate impedance sensing device, Applied Biophysics) was used to assay endothelial dysfunction in HUVECs.

Results:

We isolated exosomes from the sera of 24 healthy donors and 31 patients with PV. We found the presence of the mutated JAK2 mRNA in all samples confirming that the mutated JAK2 transcript is packaged into sera-exosomes. Yet, while healthy donors' derived exosomes carry only the wild type JAK2 mRNA, we detected both the wild type and the mutated JAK2 isoforms in patients with PV.

We showed that PV-exosomes induced thrombin generation. Then we compared PV-exosomes and healthy-individuals' derived exosomes and found that thrombin generation potential of PV-exosomes is higher in all measured...
parameters (p< 0.05 in all parameters). Furthermore, using various platelet markers (CD62, CD63, PAC1) we confirmed that PV-exosomes induced platelet activation. Together, these findings suggest that PV-exosomes may promote thrombosis directly.

Under physiological conditions the integrity of the vascular wall prevents uncontrolled thrombi. Therefore, we wondered whether PV-exosomes induce endothelial dysfunction, and in this way contribute indirectly to the pro-thrombotic phenotype. PV-exosomes were taken up by HUVEC endothelial cells in a dose- and time- dependent manner and TEER assay revealed that exosomal uptake by HUVECs induced endothelial dysfunction by breaking the tight junction between adjacent endothelial cells.

**Summary/Conclusion:**

Exosomes may act as "mini platelets" that promote thrombosis in patients with PV. PV-exosomes may promote thrombosis either directly by activating platelets and inducing thrombin generation or indirectly by inducing endothelial dysfunction.