Malignant alliances: How circulating tumor cells and their extracellular vesicles orchestrate platelet education in the bloodstream

Abstract Submitter: Zahra Eslami-Samarin, France*

Co-Authors: Luis Enrique Cortés-Hernández, Ilias Glogovitis, Keerthi Kurma, Silvia Batista, Mafalda Antunes-Ferreira, Silvia D'Ambrosi, Said Assou, Benjamin Riviere, Marion Pirel, Françoise Garima, Myron G Best, Danijela Koppers-Lalic, Thomas Wurdinger, Laure Cayrefourcq, Bruno Costa Silva, Catherine Alix-Panabières

* Laboratory of Rare Circulating Human Cells and Liquid Biopsy – University Medical Center of Montpellier, France; CREEC/CANECEV, MIVEGEC (CREES), Université de Montpellier, CNRS, IRD, Montpellier, France; European Liquid Biopsy Society (ELBS)

Background and objectives: Platelets, derived from megakaryocytes, play key roles in hemostasis, immunity, and tumorigenesis. They interact with circulating tumor cells (CTCs), promoting tumor growth, epithelial-mesenchymal transition (EMT), and metastasis. We explored the impact of CTC-platelet interactions and extracellular vesicles (EVs) on platelet activation and transcriptomic changes.

Methods: Platelets from healthy donors were exposed to thrombin or conditioned medium from colon cancer CTC lines. Morphological and protein expression changes were monitored using microscopy and flow cytometry. Platelet transcriptomic alterations were assessed by RNA sequencing after indirect co-culture. Gene expression related to EMT and cancer development in CTCs was quantified by RT-qPCR following direct co-culture with platelets. EVs isolated from CTCs were characterized by nanoparticle tracking analysis, transmission electron microscopy, and flow cytometry. EV biodistribution *in vivo* was studied by injecting labeled EVs into NOD-SCID mice, followed by microarray analysis of megakaryocytes/platelets' transcriptomes.

Results: Exposure to CTC-conditioned medium and indirect interaction with platelets induced morphological and transcriptomic changes in platelets. Gene expression related to EMT decreased in CTCs co-cultured with platelets, while mesenchymal markers remained unchanged. In contrast, genes involved in cancer invasiveness were upregulated. EVs from CTCs increased megakaryocyte numbers and induced transcriptomic changes, particularly from the aggressive CTC41.5G line. EV-treated mice showed elevated IL-6, TPO and D-dimer levels indicating increased platelet production and systemic coagulation. Higher platelet counts along with changes in megakaryocyte and platelet RNA profile were observed.

Conclusion: For the first time, we investigated the CTC-platelet cross-talk using our unique colon CTC lines. Our findings revealed that incubation with CTC-conditioned medium induced platelet aggregation and activation, supporting the hypothesis that this interaction may help preserve CTC integrity during their journey through the bloodstream. Additionally, co-culture with platelets influenced the expression of genes involved in invasiveness and EMT maintenance in CTCs. Furthermore, we highlight how CTC-derived EVs educate megakaryocytes, altering platelet function and transcriptomics and contributing to cancer-induced coagulation, likely facilitating cancer progression.

Keywords: Circulating tumor cells, extracellular vesicles, megakaryocytes, platelets, cancer, epithelial-to-mesenchymal transition (EMT), metastasis, platelet aggregation, cancer progression

Prof. Catherine Alix-Panabières, Laboratoire Cellules Circulantes Rares Humaines et Biopsie Liquide; Site Unique de Biologie - 2ème étage, 371, avenue du Doyen Gaston GIRAUD, 34295 Montpellier, France; Phone: +33 4 67 33 33 25; Fax: + 334 67 33 52 81 ; E-mail: c-panabieres@chu-montpellier.fr ; Dr. Zahra Eslami-S, Laboratoire Cellules Circulantes Rares Humaines et Biopsie Liquide; Site Unique de Biologie - 2ème étage, 371, avenue du Doyen Gaston GIRAUD, 34295 Montpellier, France; Phone: +33 4 67 33 33 24; Fax: + 334 67 33 52 81; E-mail: z-eslami-samarin@chu-montpellier.fr