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COMMENTARY

Differential effects of physiological agonists on the proteome of platelet-derived extracellular vesicles

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Abstract

Arterial thrombosis contributes to some of the most frequent causes of mortality globally, such as myocardial infarction and stroke. Platelets are essential mediators of physiological haemostasis and pathological thrombosis. Platelet activation is controlled by a multitude of signalling pathways. Upon activation, platelets shed platelet-derived extracellular vesicles (pEVs). In this Special Issue: Extracellular Vesicles, Moon et al. investigate the impact of various platelet agonists (thrombin, ADP, collagen) on the proteome of pEVs. The study demonstrates that pEVs exhibit an agonist-dependent altered proteome compared to their parent cells, with significant variations in proteins related to coagulation, complement, and platelet activation. The study observes the rapid generation of pEVs following agonist stimulation with specific proteome alterations that underscore an active packaging process. This commentary highlights the implications of their findings and discusses the role of pEV cargo in cardiovascular disease with potential novel therapeutic and diagnostic opportunities.

KEYWORDS
body fluids, cardiovascular system, clinical proteomics, coagulation, extracellular vesicles platelets, haematological disease, non-coding RNA, thrombosis

Arterial thrombosis, including myocardial infarction and ischaemic stroke, remains the leading cause of morbidity and mortality worldwide. Platelets are essential mediators of physiological haemostasis and pathological thrombosis. Platelet activation is controlled by numerous pathways, enabling a rapid response to diverse stimuli. While the aggregation response of platelets to different stimuli has been extensively studied, less is known on how different activation pathways affect the release of platelet-derived extracellular vesicles (pEVs). EVs contain lipids, proteins, and RNA and have been implicated in cell-cell communication [1]. EVs are secreted from a variety of cells. Platelets, however, are the main source of EVs in the circulation [1]. EVs derived from platelets can be identified by markers such as CD41 (integrin alpha-IIb) and CD62P (P-selectin). These receptors facilitate binding of pEVs to sites of inflammation [2] or cancer [3].

pEVs have been implicated as causal mediators in inflammatory [4] and thrombotic disorders [1]. For instance, platelets from patients with Scott's syndrome produce fewer pEVs. They have mutations in the gene encoding scramblase-1 (PLSCR1) and fail to expose phosphatidylserine on their outer membrane [5]. Moreover, pEVs have gained interest for use as drug delivery vehicles, due to their presence in the circulation and specific targeting for cellular communication [6]. Given the possible functions of pEVs in health and disease, their protein content likely varies to meet different demands. Early proteomic studies have revealed a direct proportionality between platelet protein content and

pEV size [7], as well as agonist potency and pEV protein abundance [8]. However, it was unknown how different platelet activation pathways alter the protein cargo of pEVs. Furthermore, little was known about how platelet activation impacts the pEV proteome compared to paired activated and resting platelets.

In this commentary we discuss the new data presented by Moon et al. [9], who investigate the impact of different platelet agonists on the proteome of pEVs and demonstrate that pEVs exhibit an agonist-dependent altered proteome compared to their parent cells, suggestive of an active, agonist-dependent protein packaging process. For their study, the authors used washed human platelets, removing the plasma background before mass spectrometry analysis. The authors then stimulated the washed platelets with different agonists for 30 min and collected pEVs by a centrifugation-based protocol from the plasma-free platelet releasate. Importantly, the authors also collected the activated platelets, as well as resting platelets from the same blood donation, allowing for a paired analysis between these three sample types using proteomics.

Interestingly, pEV proteomes were distinct to their platelet parent proteome, suggestive of active protein cargo selection. The core EV proteins, including proposed universal EV markers, such as synthenin-1 and CD63, were enriched in pEVs compared to paired platelets, whilst the majority of cellular proteins were enriched in platelets compared to pEVs. Other proteins enriched in pEVs belonged to proteins of the complement and coagulation cascade including antithrombin, plasminogen and prothrombin. The authors then compared the proteomes of pEVs released by platelets activated by different agonists. Based on their protein composition, pEVs following thrombin and thrombin/collagen stimulation clustered together, whereas pEVs following ADP or collagen activation clustered separately, reflecting agonist potency. pEVs were collected from platelets within 30 min of stimulation. Thus, platelets package proteins into pEVs without *de novo* protein synthesis. Using SILAC (Stable Isotope Labeling by Amino acids in Cell culture) labelling and mass spectrometry, we have previously demonstrated that *de novo* protein synthesis in platelets is not a major contributor to platelet protein content [11].

One major strength of the study is its paired experimental design allowing for direct comparisons between non-activated platelets, activated platelets and pEVs from the same donors. Proteins with lower abundance in activated platelets compared to resting platelets were found to be enriched in pEVs. Another strength is the use of plasma-free platelet releasate, thus avoiding a contamination with EVs from other sources. Among the study's limitations is its small sample size ($N = 5$), but the use of healthy volunteers excludes cofounding by medication or co-morbidities. Additionally, centrifugation-based EV isolation protocols can be contaminated with non-EV particles, in particular lipoproteins, which are similar in size and density as EVs. Again, lipoprotein contamination of pEVs will be reduced in plasma-free platelet releasate [1]. Future studies should evaluate vesicle composition, that is, differences in the protein cargo between exosomes and microvesicles. Moreover, in acute diseases such as myocardial infarction the platelet proteome may also be modified by horizontal transfer from other cells such as neutrophils [11]. From a clinical perspective, understanding the mechanisms that control the formation and composition of pEVs may be useful for diagnostic and therapeutic purposes [6]. Conversely, the pEV proteome may provide insights into disease outcomes and platelet reactivity [10]. The precise mechanisms of protein packaging into pEVs remain unknown and will need to be explored in future studies.

Taken together, the data by Moon et al. [9] demonstrate that pEV protein composition is an agonist-dependent process that does not rely on *de novo* protein synthesis. The finding that the activation pathway used to stimulate platelets affects the pEV proteome is an important insight and suggests that pEVs released by different agonists might have distinct biological functions. Future studies are needed to dissect the molecular mechanisms of pathway-dependent pEV release. In addition to proteins, pEVs also carry non-coding RNAs [12], which may exhibit similar agonist specificity. A combined protein and RNA analysis would be of interest for future studies.

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CONFLICT OF INTEREST STATEMENT

C.G. reports no conflict of interest. M.M. filed and licensed patent applications on miRNAs as biomarkers.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current stydy.

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REFERENCES

1. Puhm, F., Boilard, E., & Machlus, K. R. (2021). Platelet extracellular vesicles. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *41*, 87–96.
2. Kuravi, S. J., Harrison, P., Rainger, G. E., & Nash, G. B. (2019). Ability of platelet-derived extracellular vesicles to promote neutrophil-endothelial cell interactions. *Inflammation*, *42*, 290–305.
3. Michael, J. V., Wurtzel, J. G. T., Mao, G. F., Rao, A. K., Kolpakov, M. A., Sabri, A., Hoffman, N. E., Rajan, S., Tomar, D., Madesh, M., Nieman, M. T., Yu, J., Edelstein, L. C., Rowley, J. W., Weyrich, A. S., & Goldfinger, L. E (2017). Platelet microparticles infiltrating solid tumors transfer miRNAs that suppress tumor growth. *Blood*, *130*, 567–580.
4. Boilard, E., Nigrovic, P A., Larabee, K., Watts, G. F. M., Coblyn, J. S., Weinblatt, M. E., Massarotti, E. M., Remold-O'donnell, E., Farndale,

R. W., Ware, J., & Lee, D. M. (2010). Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science*, *327*, 580–583.
5. Sims, P. J., Wiedmer, T., Esmon, C. T., Weiss, H. J., & Shattil, S. J. (1989). Assembly of the platelet prothrombinase complex is linked to vesiculation of the platelet plasma membrane. *Journal of Biological Chemistry*, *264*, 17049–17057.
6. Hu, C. M., Fang, R. H., Wang, K. C., Luk, B. T., Thamphiwatana, S., Dehaini, D., Nguyen, P., Angsantikul, P., Wen, C. H., Kroll, A. V., Carpenter, C., Ramesh, M., Qu, V., Patel, S. H., Zhu, J., Shi, W., Hofman, F. M., Chen, T. C., Gao, W., … Zhang, L. (2015). Nanoparticle biointerfacing by platelet membrane cloaking. *Nature*, *526*, 118–121.
7. Dean, W. L., Lee, M. J., Cummins, T. D., Schultz, D. J., & Powell, D. W. (2009). Proteomic and functional characterisation of platelet microparticle size classes. *Thrombosis and Haemostasis*, *102*, 711–718.
8. Milioli, M., Ibáñez-Vea, M., Sidoli, S., Palmisano, G., Careri, M., & Larsen, M. R. (2015). Quantitative proteomics analysis of platelet-derived microparticles reveals distinct protein signatures when stimulated by different physiological agonists. *Journal of Proteomics*, *121*, 56–66.
9. Moon, M J., Rai, A., Sharma, P., Fang, H., Mcfadyen, J. D., Greening, D. W., & Peter, K. (2024). Differential effects of physiological agonists on the proteome of platelet-derived extracellular vesicles. *Proteomics*, e2300391.
10. Gutmann, C., Joshi, A., & Mayr, M. (2020). Platelet "-omics" in health and cardiovascular disease. *Atherosclerosis*, *307*, 87–96.
11. Joshi, A., Schmidt, L. E., Burnap, S. A., Lu, R., Chan, M. V., Armstrong, P. C., Baig, F., Gutmann, C., Willeit, P., Santer, P., Barwari, T., Theofilatos, K., Kiechl, S., Willeit, J., Warner, T. D., Mathur, A., & Mayr, M. (2022). Neutrophil-derived protein S100A8/A9 alters the platelet proteome in acute myocardial infarction and is associated with changes in platelet reactivity. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *42*, 49–62.
12. Gutmann, C., Joshi, A., Zampetaki, A., & Mayr, M. (2021). The landscape of coding and noncoding RNAs in platelets. *Antioxidants & Redox Signaling*, *34*, 1200–1216.

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