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Platelet-derived microparticles – an updated perspective

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ABSTRACT

Platelet-derived microparticles (PMP) are a heterogeneous population of vesicles (<1 μm) generated from the plasma membrane upon platelet activation by various stimuli. They are a discrete population differing from the exosomes which originate from the intracellular multivesicular bodies. PMP also differ from the microparticles derived from megakaryocytes despite the presence of several identical surface markers on the latter. The molecular properties and the functional roles of the PMP are beginning to be elucidated by the rapidly evolving research interest, but novel questions are simultaneously raised. This updated perspective discusses the most recent highlights in the PMP research in context with the methodological problems and the paradoxical role of the PMP in health and disease.

Abbreviations

PMP: platelet-derived microparticle(s)
MP: microparticle(s)
PS: phosphatidylserine

Introduction

The awareness and the research of platelet-derived microparticles (PMP) have evolved rapidly during the past two years. While the publications of the PMP properties and effects are revealing novel functions *in vivo*, they also simultaneously define new questions. This update will focus on the most exciting developments in the PMP field mainly within the past two years. The emphasis is to discuss the origin of PMP, the pitfalls of current methodology in PMP isolation and detection, and finally to compare the paradoxical role of PMP in physiological and pathological settings. While this update does not constitute a comprehensive view on PMP, several excellent reviews of microparticles (MP) have been published in 2009 and 2010, and the interested reader is invited to turn to them for more extensive accounts of MP in general [1–3] and for a clinical perspective [4–15].

The origins of PMP in plasma

Microparticles are shed from several cell types including leukocytes, erythrocytes, endothelial cells and various cancer cells. This list is still expanding with the recent additions of various cells in the central nervous system [10] and adipocytes [16]. Thus, it is not far-fetched to say that "possibly all eukaryotic cells generate MP after stimulation or apoptosis" [3], which promotes the concept of MP generation as a truly universal mechanism of intercellular communication.

It has long been thought that 70–90% of MP in plasma are derived from activated platelets in comparison to other MP-generating cells which contribute to the total pool. In 2009, Flaumenhaft et al. showed that megakaryocytes also generate CD41/CD61-positive MP which differ from the PMP in that they do not express the markers of granule fusion (CD62 or LAMP-1) or cytoskeletal degradation (degraded filamin A) [17]. These phosphatidylserine (PS)-positive particles were generated by both murine and human megakaryocytes in cell culture, and they were found to be the predominant "PMP" population in the plasma of healthy humans. Although the concept of the majority of "PMP" originating from megakaryocytes instead of platelets certainly needs further validation, it was also recently shown that the CD61-positive MP population largely disappeared from circulation after bone marrow irradiation, whereas the PMP positive for CD62 remained [18], in support of the finding of a significant presence of megakaryocyte-derived MP in normal plasma.

It is now accepted that PMP (and other MP) mediate intercellular transfer of bioactive molecules such as lipids, surface receptors and even enzymes [19–22]. More importantly, these molecules mediate changes in the recipient cell functions (e.g. CXCR4, CD41). If the resulting membrane fusion distributes cell-specific identification markers from one cell type to another, it confounds the identification of origins of the MP, which is most often determined by the binding of antibodies for a single parent marker (see below). In the past, it has been suggested that "hybrid MP" – which would carry markers for both platelets and, e.g., monocytes – could be generated when two different MP classes fuse together, or when, e.g., monocyte-derived MP become incorporated into platelets, which then vesiculate in turn [23]. Such MP fusions could

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explain the conflicting reports on the origin of tissue factor (TF) in circulation [24,25]. If such fusion MP existed, they could offer a carefully controlled means of providing active TF. Regarding the identification of the cellular origins of MP, the possibility of such fusions presents a novel challenge.

The molecular basis of the formation of membrane-derived MP is less well-understood than exosome formation or apoptotic blebbing. Although the rise of intracellular Ca^{2+} is an established key point in the signal transduction leading to the loss of lipid asymmetry and the membrane vesiculation, some of the other paradigms of the mandatory signaling may need to be rethought. For instance, degradation of the cytoskeleton by proteases seems to be dispensable for the megakaryocyte-derived MP [17], and not all PMP express PS (see later). These observations raise questions about how the different platelet-activating conditions affect the PMP formation, and how they relate to the heterogeneity of the PMP pool. Recently, CD9, an abundant platelet surface tetraspanin of unknown function, was shown to be important for the generation of thrombin and collagen-induced PMP together with CD41/61 [26], whereas the generation of high shear stress-induced PMP was inhibited by antibodies against CD42b irrespective of filamin A degradation [27]. However, the co-inhibition of CD61 (by abciximab) with the CD42b blocking antibodies further attenuated the PMP generation under high shear rate conditions, and also partially inhibited filamin degradation. Thus, it seems that despite the apparent dissimilarity in the PMP generation pathways, some mechanisms or participating receptors may be convergent. The molecular and the cell-biological mechanisms of MP generation may give us the final answer for their function, and hence more studies which compare the signal transduction events of microvesiculation by different agonists are instrumental.

Isolation and detection dilemma

Increasing evidence enforces the need for standardization of both PMP isolation and analysis, and this task is currently being addressed by the Scientific Standardization Committee of the International Society on Thrombosis and Haemostasis (www.isth.org). The first steps for setting guidelines for the use of flow cytometry in the PMP enumeration have been taken [28], and the ISTH SSC Collaborative Workshop demonstrated that it was possible to reproducibly analyze PMP samples in different laboratories [29]. However, the preanalytical stages of PMP isolation also need to be addressed. The PMP for analysis are obtained by recovering samples from platelets/platelet remnants by various centrifugation protocols. The centrifugation steps tend to differ, and only in a few studies has the absence of platelets/platelet remnants actually been verified, although freeze–thawing of platelets artificially generates PMP [30]. Furthermore, other preanalytical steps (anticoagulant, delay in processing, transport, storage, defrosting) should also be carefully considered ([31] and the 56th Annual Scientific Standardization Meeting Minutes). Combined with the variation in the analytical methods, this makes the synthesis of information from parallel PMP studies very difficult, if not impossible, and hence universal guidelines for both the isolation and the detection of MP are urgently needed.

Another confounding factor in need of methodological consideration is the presence of exosomes in the samples that are described as PMP [2]. Activated platelets also secrete exosomes [32] which form a more uniform vesicle population than the heterogenous PMP. Exosomes are considered to essentially differ from the MP by size (~40–100 nm), density, and molecular content (e.g. tetraspanin enrichment, reviewed in [33]). Exosomes originate from the multivesicular bodies within cells and their secretion is both constitutive and activation-dependent, whereas PMP are derived from the plasma membrane after platelet activation. Unless differential centrifugation steps or filtration with different cut-off membranes have been performed, all the PMP samples also contain exosomes. Thus, any conclusions on the role or the properties of PMP may need to be attributed to the exosome population instead. For example, all the PMP proteome studies to date have not separated the exosomes from the PMP analyte. A collective term of "microvesicles" could be adopted for both the MP and the exosomes, as suggested recently [33].

The widely acknowledged difficulty with the use of flow cytometry in the detection and quantification of PMP is that most present-day instruments obtain data ineffectively on the smaller sized PMP (<500 nm), a property of the laser wavelengths [34]. The arrival of new-generation flow cytometers and standardization of calibration with polystyrene beads are stretching the lower cut-off limit to 300 nm, and maybe even lower, but the results from alternative technologies, e.g. atomic force microscopy (AFM), suggest that flow cytometry still results in gross underestimation of the quantity (~1000× difference) and the size of PMP. Yuana et al. detected CD41-positive PMP with the mean calculated diameter of 50 nm (range 10–475 nm) in AFM [35].

Analysis by flow cytometry may also possibly underestimate other properties of the PMP. Currently, the identification of PMP is based on size (<1 μm) and the binding of fluorescent-labeled antibodies to markers characteristic for the parental cell type. For PMP, the most frequent markers are the abundant surface receptors CD41, CD61or CD42b, or the platelet activation-dependent CD62. The use of any one of these markers individually fails to detect all of the PMP due to the qualitative differences in the PMP subpopulations, as shown by a recent proteomic and functional study of the PMP size fractions [36]. In this study, thrombin- and collagen-induced PMP were segregated into arbitrary size classes by gel filtration chromatography, their protein signatures were analyzed by proteomics, and functional properties were assigned by PFA-100. The subclasses were shown to differ in size, protein content and thrombogenic potential, which highlights the importance of the accurate identification of the PMP (sub)populations, especially in clinical studies.

Since the exposure of PS is tightly associated with MP formation, PS is used for PMP detection by Annexin V, or lately by lactadherin (milk fat globule-epidermal growth factor 8). Lactadherin-binding is independent of the presence of Ca^{2+}, and has a higher affinity for even small amounts of PS (<1%) in contrast to Annexin V, which makes it a superior tool [37]. Interestingly, lactadherin, secreted by macrophages and adipocytes, was also detected on the surface of PMP in circulation [38]. Lactadherin-deficient mice had increased levels of PMP, which suggests a role for lactadherin in the systematic clearance of the PMP by phagocytosis. It is noteworthy that the capture of the plasma PMP by PS, e.g. in ELISA, will include also MP from other cell types. More importantly, it seems that not all PMP are positive for PS [39,40], making the PS-positive PMP another subclass within the total PMP population.

Novel techniques such as impedance-based flow cytometry [41], electrochemical impedance spectroscopy [42], atomic force microscopy [35] and dynamic light scattering [43,44], do offer improvements in the PMP detection, particularly in their capacity to detect all the MP, irrespective of their size, but these techniques also have severe limitations due to the scarcity of available instrumentation and the lack of molecular identification provided by the methods which do not utilize antibody detection. Other technologies based on light scattering, e.g. electrophoretic quasi-elastic light scattering [45] and nanoparticle tracking analysis [46], are being developed to allow molecular detection. However, for most studies, especially with clinical samples, flow cytometry or solid-phase capture are still likely to be the methods of choice for the near future. Also, if all the above mentioned aspects of