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Platelet proteomics in cardiovascular diseases

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ABSTRACT

In recent years, platelet proteomics has been applied successfully to the study of cardiovascular diseases (CVDs). It is very well known that platelets play a pivotal role in the pathophysiological mechanisms underlying many CVDs, especially acute coronary syndromes (ACSs), since they are implied in thrombus formation after atheroma plaque rupture. This is the reason why molecules involved in platelet activation and aggregation are primary targets for treatment of ACSs. Many efforts are aimed at finding drugs that inhibit platelet activation; however it is difficult to separate the therapeutic benefits from harmful effects because pathological and physiological functions of platelets are due to the same mechanisms. Given that platelets lack a nucleus, proteomics is regarded as an ideal method to approach their biochemistry. Current platelet proteomic studies are focusing on the identification of platelet molecular and functional changes in normal and pathological states, enriching the comprehension of platelet biological function, and screening for new biomarkers and antiplatelet agents. In the present article, we introduce the reader to platelet biology and function, and revise recent advances in platelet proteomics applied to the study of CVDs, including a special emphasis on sample preparation requirements for proteome analysis of platelet clinical samples.

Contents

Abbreviations: ACS, acute coronary syndrome; ADP, adenosine diphosphate; ANG, angiotensin; ARB, angiotensin II receptor blocker; ASA, Acetyl Salicylic Acid; ATP, adenosine triphosphate; CAD, coronary artery disease; CRP, collagen-related peptide; CVD, cardiovascular disease; GPS, gray platelet syndrome; GPVI, glycoprotein VI; IPA, ingenuity pathway analysis; LDL, low-density lipoprotein; MK, megakaryocyte; MP, microparticle; MS, mass spectrometry; MV, microvesicle; NO, Nitric oxide; NP-40, Nodinet P-40; NSTEMI, Non ST-Segment Myocardial Infarction; PCI, percutaneous coronary intervention; PFP, platelet-free plasma; PGI2, prostaglandin I2; PRP, platelet-rich plasma; PS, phosphatidyl serine; SA, stable angina; SCAD, stable coronary artery disease; STEMI, ST-Segment Myocardial Infarction; TCA, trichloroacetic acid; TRAP, thrombin receptor activating peptide; TXA2, thromboxane A2; UA, unstable angina.

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1. Introduction

1.1. Platelet definition, biogenesis and subpopulations

Platelets are specialized annucleate mammalian blood cells averaging 2–3 μm in diameter and 0.5 μm in thickness. They have discoid shape under normal physiological conditions, and their half-life is 10 days in peripheral blood. Platelets derive from megakaryocytes (MKs), which are unique polyploid hematopoietic cells that are found only in mammals and are specialized to produce and release platelets into the blood circulation. Although MKs arise in the bone marrow, they can migrate and nest at non-marrow sites, reason why platelet biogenesis (thrombopoiesis) has been proposed to take place not only in the bone marrow but also in lungs and blood. Different stages of platelet development have been observed in all these three locations.

For a long time it has been questioned whether the MKs present in the bone marrow, in the lungs or in the blood, are responsible for the greater production of platelets. For example, the lungs are reservoirs for megakaryocytes and platelets, releasing them in response to certain stimuli. Platelets continuously circulate through pulmonary vessels, contributing to lung defense, disease, and remodeling, being the effectors of injury in a variety of pulmonary disorders and syndromes [1]. The estimated contribution of pulmonary MKs to total platelet production remains unclear, but in theory platelets and MKs may reach the pulmonary circulation completing their development into platelets in lung capillaries.

Among the two critical functions of platelets, which are adhesion at site of vascular injury and promotion of thrombin generation, it is assumed that all platelets are equally able to satisfy the former one. Nevertheless, in the last decade some studies suggest that some platelets have a greater ability to promote thrombin generation. Within a thrombus, there are clearly distinguishable platelets with different surface properties. Different platelet populations and their functions were recently reviewed by Heemskerk et al. [2], and are briefly described below.

Close to collagen fibers, there is a population of highly activated platelets arranged as patches around the thrombus. These platelets, known as procoagulant platelets, have a rounded structure, expose PS in their surface, have a sustained calcium-induced morphology, the ability to bind coagulation factors, and produce procoagulant microvesicles (MVs).

Coated-platelets are another subpopulation formed after platelet stimulation with collagen plus thrombin, although some studies demonstrated that an indistinguishable subpopulation of cells can be produced by stimulation with other agonists pairs or even with single agonists [3–6]. Coated-platelets show high levels of several α-granule procoagulant-proteins on their surface (i.e. FV, fibrinogen, thrombospondin, vWF), expose high levels of surface PS, are functional in their ability to bind FXa and generate an active prothrombinase complex, bind serotonin-conjugated proteins, and also shed MVs. The physiological significance of coated-platelets is still unknown, although it is speculated that they could be significant contributors to thrombotic process. The potential clinical relevance of coated-platelets in hemostasis may be the contribution to a hemorrhagic or thrombotic phenotype. Thus, a low level of coated-platelets is correlated with the appearance of spontaneous intracerebral hemorrhages [7] and with bleeding diathesis [8], while a high amount of them seems to correlate with transient ischemic attack and ischemic stroke [9,10], and with patients with a history of arterial or venous thrombosis [11]. Coated-platelets also appear to have an impact on the bleeding phenotype in severe hemophilia [12] among other diseases.

In a thrombus, there are also aggregate-forming platelets. These platelets are characterized by the presence of active αIIbβ3 in their surfaces and fibrinogen binding to this receptor. Under coagulant conditions, procoagulant platelets produce FXa and thrombin outside the platelet plug, whereas aggregating and clot-retracting platelets are responsible for plug consolidation and clot retraction.

A final category of platelets are apoptotic and necrotic platelets, characterized by an apoptotic PS exposure, which appears to rely on caspase activation. Although several in vitro studies have reported that these platelets have a coagulant potential and support thrombin generation, their role in physiological conditions is questionable, as they are rapidly taken up by scavenging cells present in the blood circulation. Moreover, it has been suggested that apoptotic and necrotic platelets are activated by a necrotic cell death pathway [13].

1.2. Platelet function

Platelets main function in hemostasis was recognized more than a century ago [14]; indeed, low platelet count is responsible for hemorrhage and prolonged bleeding time, even despite normal coagulation [15]. Nowadays some other non-hemostatic crucial biological functions are known in relation to platelets, such as inflammation [16], immunity [17,18], malignancy [19] and maintenance of vascular integrity [20].

1.2.1. Role of platelets in hemostasis, thrombosis and cardiovascular disease

Under normal conditions, endothelial cells of the vessel walls synthesize prostaglandin I2 (PGI2) and nitric oxide (NO) as inhibitors of platelet function, acting synergistically [21–23]. They have also ecto-ADPase (CD39) activity, which metabolizes ADP (platelet activator) to adenosine (platelet inhibitor) [24,25]. These three mechanisms working coordinately inhibit platelet activation. When the endothelium is damaged, these mechanisms are altered and subendothelial matrix is exposed undergoing potent platelet-activation and shape change, leading to platelet plug formation in order to avoid excessive bleeding. Thrombus generation starts with a platelet plug formation followed by stabilization of this plug through fibrin deposition (coagulation). Primary platelet adhesion