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Thrombus lysis is the consequence of a restricted number of reactions localised on the surface of fibrin and cell membranes. A functional defect or an insufficient fibrinolytic response may lead to thrombosis with severe or fatal clinical consequences. Despite this clinical exigency and a real progress in the knowledge of the different components of this system (plasminogen activators, inhibitors and receptors) including structure-function relationship unveiled by the crystal structure of plasminogen, the functional evaluation of fibrinolysis still remains a challenge in haemostasis. The absolute requirement of a template for molecular assembly of plasminogen and its activators (tPA or uPA) restricts the formation of plasmin onto the surface of fibrin and cells. In contrast, during fibrinolysis plasmin and tPA released from the clot are immediately neutralised by their respective inhibitors, α_2-antiplasmin and plasminogen activator inhibitor 1, PAI-1. It is therefore almost impossible to detect fibrinolytic activity in plasma with current methods or to measure the degree of fibrinolysis directly on clots unavailable in a clinical setting.

Interestingly, it was recently discovered that circulating membrane microvesicles, which behave as messengers of cell activation, might be indicators of the fibrinolytic response to an inflammatory or prothrombotic process.[1, 2] These fibrinolytic microvesicles transport at their membrane the plasminogen activators expressed by the cell of origin: tPA from endothelial cells and uPA from leukocytes.[3] These microvesicles generate plasmin *in situ* upon binding of plasminogen to carboxy-terminal lysine residues of membrane receptors. Thus, co-assembly of plasminogen and its activator onto the same surface is required to trigger the fibrinolytic or proteolytic process classically described on cell membranes and fibrin.

We recently discovered that moving surfaces such as microvesicles might also participate in a new mechanism of plasmin formation requiring a cross-talk between two different surfaces.[2] In this fibrinolytic cross-talk, one of the surfaces bearing uPA/uPAR (leukocyte or its microvesicles) can recognize and activate plasminogen carried by the other surface (platelets,

fibrin or extracellular matrix). Plasminogen thus bound adopts an open conformation that is readily transformed into plasmin. Recently published studies[4-6] are in agreement with these hitherto unknown fibrinolytic pathway and potentially novel biomarkers in clinical practice.

Relevance of the fibrinolytic cross-talk mechanism

This novel mechanism of plasmin formation at the surface of platelets, extracellular matrix or fibrin by microvesicles bearing uPA raises the question of its involvement in different pathophysiological situations:

Fibrinolysis: recanalisation of occluded vessels

Platelets and derived microvesicles do not bear plasminogen activators. However, they can immobilise plasminogen on their surface via carboxy-terminal lysine residues-dependent interactions.[7] Platelets may thus contribute to increase the concentration of plasminogen within the clot. Microvesicles bearing uPA could then cross-talk with platelet-bound plasminogen thus allowing *in situ* plasmin formation and re-canalisation of an occluded vessel. Similarly, activation of plasminogen bound to fibrin by leukocytes bearing uPA plays a role in endogenous fibrinolysis.[6]

Cell migration and angiogenesis

Aside from its fibrinolytic function, plasmin formation by the uPA/uPAR system is involved in tissue remodelling and plays a critical role in cell migration and angiogenesis. The ability of microvesicles to generate plasmin influences and modulates the repair process of endothelial progenitor cells. Small amounts of microvesicles bearing the uPA/uPAR system promote cell migration and angiogenesis

whereas at high concentrations excess plasmin leads to matrix degradation, decreased cell adhesiveness and finally apoptosis.[1]

Dissemination of cancer cells

Platelet microvesicles may promote metastasis and angiogenesis, [8] and high amounts of uPA/uPAR were associated with matrix degradation and loss of cell adhesion in advanced metastatic cancers.[9] It is interesting to note that the described fibrinolytic/proteolytic cross-talk mechanism is only possible in the presence of uPA, which has been found on microvesicles emitted by cancer cells.

Conclusion and potential applications

The structure and function of the plasminogen activation system and its role in the maintenance of haemostasis and thrombosis prevention is now well established. However, detection of a dysfunction of this system remains a major challenge in clinical practice. Actually, plasminogen activators circulate at extremely low concentrations as inactive complexes with PAI-1 whereas active plasminogen activators are exclusively located on the cell membrane or fibrin. Since most measurements performed in plasma or its euglobulin fraction do not take into account the contribution of surface-bound plasminogen activators, it is impossible to quantify a lack of tPA or uPA activity that may be the cause of a fibrinolytic insufficiency. The recent discovery of fibrinolytic microvesicles and their role in fibrinolytic cross-talk, opened up new perspectives. We propose that the fibrinolytic activity conveyed by microvesicles could be the real source of fibrinolysis in circulating blood. These microvesicles would act on and within the clot, thus explaining the lack of systemic fibrinolysis. We suggest that the fibrinolytic activity of endothelial and leukocyte microvesicles compensates locally the activity of procoagulant microvesicles. Spontaneous

re-canalization in 15-20% ST-segment elevation myocardial infarction,[10] could be thus explained by fibrinolytic microvesicles counterbalancing procoagulant microvesicles. At the opposite, a failure of timely fibrinolytic response by microvesicles would result in persistence of occluding thrombi. Accordingly, the functional balance between these two types of microvesicles would result in a physiological haemostatic response, while the lack of fibrinolytic microvesicles may allow thrombus formation. Within this context, it will be possible to use microvesicles as vectors of fibrinolysis and pericellular proteolysis. The existence of a hemorrhagic syndrome (Quebec platelet disorder) caused by profibrinolytic platelets having an abnormal expression of uPA is consistent with this hypothesis.[11] Transgenic mice that express uPA in their platelets are resistant to arterial thrombosis and transfusion of these platelets to control mice prevents occlusive arterial thrombi formation.[12] Similarly, addition of fibrinolytic microvesicles to plasma or euglobulins decreases the microplate clot lysis time in a concentration-dependent manner (unpublished results). Although the venous occlusion test induces the release of fibrinolytic microvesicles, the majority of fibrinolytic assays including the euglobulin clot lysis time, exclude microvesicle-bound plasminogen activators (unpublished results). Thus, development of a new test for the detection of fibrinolytic microvesicles directly in plasma is promising as it will bring a new light to both the pathophysiology of fibrinolysis and the management of thrombosis in clinical practice.[13]

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