Fibrinolysis, new concepts and new mechanisms: fibrinolytic microvesicles and fibrinolytic crosstalk

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Fibrinolysis, new concepts and new mechanisms: fibrinolytic microvesicles and fibrinolytic crosstalk

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ABSTRACT

Thrombus lysis is the consequence of a restricted number of reactions localised to the surface of fibrin. A functional defect or an insufficient fibrinolytic response may lead to thrombosis with severe or fatal clinical consequences, e.g. myocardial infarction and ischemic stroke. Despite this clinical exigency and a real progress in the knowledge of the different components of this system (plasminogen and its activators, inhibitors and receptors), its functional evaluation still remains a challenge in haemostasis. The absolute requirement of a template for molecular assembly of plasminogen and its activators (tissue- and urokinase-type plasminogen activators: tPA and uPA) restricts the formation of plasmin and protects its activity onto the surface of, respectively, fibrin and cells. In contrast, plasmin and tPA released from the clot during its lysis are immediately neutralised by their respective inhibitors a_2-antiplasmin and plasminogen activator inhibitor 1, PAI-1). It seems therefore almost impossible to detect fibrinolytic activity in plasma with methods currently in use. Because of its unavailability, it is also impossible to measure the degree of fibrinolysis directly on the clot. Notwithstanding, it was recently discovered that circulating membrane microvesicles might be indicators of the fibrinolytic response to an inflammatory or prothrombotic process. These cell-derived fibrinolytic microvesicles bear at their membrane the plasminogen activators expressed by the parent cell: tPA from endothelial cells and uPA from leukocytes. These molecules are localised at the membrane surface and have the capacity to activate plasminogen into plasmin *in situ*. Moreover, it was recently discovered that these microvesicles might participate in a new mechanism of plasmin formation requiring a cross-talk between two different surfaces. In this fibrinolytic cross-talk one of the surfaces bear plasminogen (fibrin, extracellular matrix or platelets) whereas the other surface carry the plasminogen activator, typically leukocyte-derived microvesicles bearing uPA. These new actors and concepts in plasminogen activation represent hitherto unknown pathways in our comprehension of fibrinolysis and potential novel biomarkers in clinical practice.

The fibrinolytic activity of the intravascular compartment is a major mechanism of defense against thrombosis. It allows specific lysis of excess fibrin formed after vascular injury in order to restore vascular integrity and blood flow. Its effectiveness depends on the simultaneous functioning of the fibrin network as (a) a support of the hemostatic clot, (b) a surface for the assembly of a ternary complex with plasminogen and tissue activator (tPA), (c) a surface for plasminogen activation and (d) a substrate for plasmin.[1]

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The tPA is synthesized and released by the endothelium in response to a number of stimuli.[2] Released into contact with the clot, it binds to fibrin but can also generate plasmin on the endothelial surface.[3] Other cellular components involved in thrombus formation are also involved in its dissolution. For instance, leukocytes that form aggregates with platelets release a second type of plasminogen activator: urokinase (uPA), which can, under certain conditions, activate plasminogen bound to fibrin.[4] However, the activation of plasminogen by uPA occcurs primarily at the cell membrane.[5] If the membrane of endothelial cells and leukocytes behave as surfaces for the production of plasmin, the membrane of platelets play an important role as a source of plasminogen within the thrombus.[6] Platelets can also develop a regulatory activity by releasing PAI-1, the major inhibitor of plasminogen activators.[7]

Recently, we have shown that beyond the participation of cells, a similar mechanism of activation of plasminogen was present at the membrane of microvesicles from the cell line HMEC-1 (human microvascular endothelial cells).[8]

Moreover, more recent studies have demonstrated the existence of a new mechanism for the formation of plasmin, the fibrinolytic cross-talk, requiring a first surface bearing plasminogen and a second suface bearing the plasminogen activator uPA.[9] The formation of plasmin on fibrin or on the cell membrane is therefore built on the close relationship between the molecular conformation of the plasminogen adsorbed onto a surface and its recognition by activators immobilized on the same surface or on a moving surface.

These two new mechanisms, the fibrinolytic activity of membrane microvesicles and the fibrinolytic cross-talk, represent new pathways for fibrinolysis. They will be analyzed in this review after having defined the role played by conformational changes of plasminogen in the mechanism of plasmin generation.

Plasminogen structure and conformational changes

This 92 kDa glycoprotein consists of 791 amino acids (Asn791-Glu1, Glu-plasminogen) that are grouped into five modules called "kringle" and a catalytic region. These modular elements are preceded by an amino-terminal peptide (Glu1-Lys77) (Figure 1A, Table 1). Kringles 1 and 4 contain binding sites for lysine residues (LBS, lysine binding site) of fibrin that allow interaction and effective binding of plasminogen. Kringle 5 contains modified LBS with affinity for the lysine residues of the amino-terminal peptide that promotes the adoption of a closed spiral conformation (Figure 1B).[10] This plasminogen closed form predominates in the circulation. The release of the amino-terminal peptide after cleavage by plasmin characterizes a truncated open conformation known as Lys-plasminogen. An open form also occurs when the LBS is occupied by lysine analogues such as epsilon aminocaproic acid (e-ACA) or tranexamic acid (TXA).[11] Indeed, invalidation of the LBS function by these molecules prevents interaction between the kringle 5 and the amino-terminal peptide. Invalidation of the LBS function by these agents also prevents binding of plasminogen to fibrin and thus its activation by tPA. Surprisingly, it was found that the open form (Figure 1B) induced by low doses of EACA is recognized and converted into plasmin by uPA. By homology, it is widely accepted that the direct interaction of native plasminogen (Glu-plasminogen) with the lysine residues of fibrin or membrane glycoproteins leads to a transition from the closed form to the open form. This form is efficiently activated by tPA linked to fibrin or uPA bound to its receptor. In summary, the binding of plasminogen to fibrin or cell membranes and its transition into the open form are necessary conditions for its transformation into plasmin by plasminogen activators located nearby. The

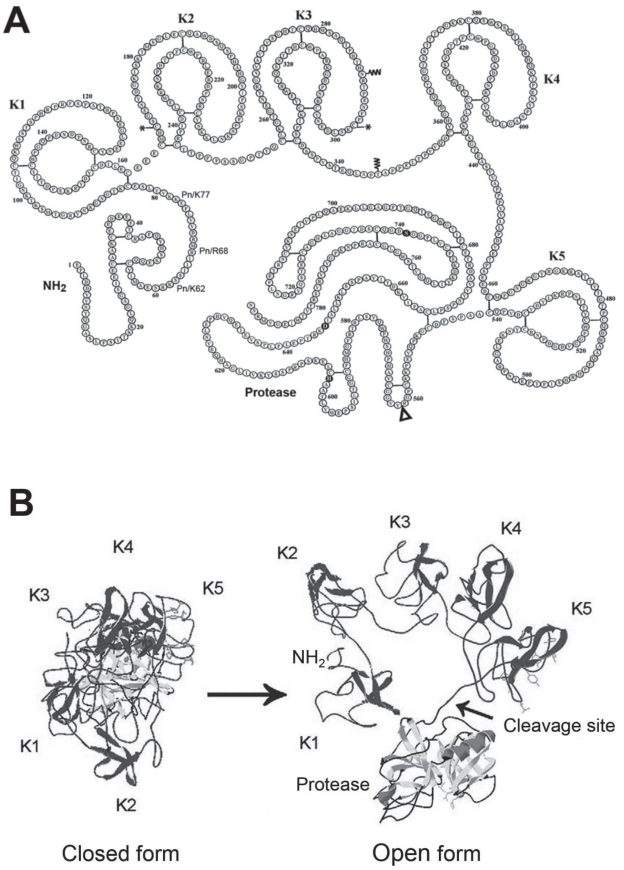

Figure 1. Structure and conformation of plasminogen.
A. Amino-acids sequence and secondary structure of plasminogen. The 791 amino-acids of Glu-plasminogen (Glu^{1}-Asn^{791}) are assembled in structural modules: an aminoterminal (NH2) peptide followed by 5 kringle modules (K) and a serine-protease region containing the amino acids of the active site (black circles). The site of cleavage by the activators (Arg^{561}-Val^{562}) is indicated by the open triangle. The cleavage by plasmin at the NH2-terminal peptide (Pn/K^{77}, Pn/R^{68}, Pn/K^{62}) results in the truncated form Lys^{77}-Asp^{791}. Original figure: http://www.chem.cmu.edu.groups/Llinas/images/res/res-kringle-PLASMIN.gif
B. The closed form (interaction between the aminoterminal peptide and kringle 5) is the main circulating conformation. The open form is adopted upon binding of plasminogen to C-Lys terminal residues of fibrin or membrane glycoproteins. Original figure (Lähteenmäki K, Edelman S, Korhonen TK. Trends in Microbiology 2005, 13:79) reproduced with permission from Elsevier Limited.

Table 1. Main components of the plasminogen activation system.

	Plasminogen	*Plasmin*	*suPAR*	*sc-uPA → tc-uPA*	*tPA*
Plasma concentration	0.12- 0.18 mg/mL	0	< 1 ng/mL	3.6 ± 0.9 ng/mL	7.5 ± 2.5 ng/mL
Molar concentration	1.5 to 2 µM	0	< 1.5 pM	50 to 85 pM	70 to 140 pM
Molecular mass	92kDa	84kDa	65kDa	54kDa	70kDa
Cleavage site	Arg^{561}-Val^{562}	-	-	Lys^{158}-Ile^{159}	Arg^{275}-Ile^{274}
Modules	P-K_{1-5}-SP	K_{1-5}-SP	D_{1-3}	EGF-K_1-SP	F-EGF-K_{1-2}-SP
Polypeptide sequence	1 chain	2 chains	1 chain	1 chain → 2 chains	1 chain / 2 chains
Enzyme activity	-	++++	-	+/- → ++++	++++ / ++++

suPAR: uPAR soluble; P: N-terminal peptide; K: kringle domaine; SP: serine protease; D: LU (Ly-6 uPAR) module; EGF: epidermal growth factor-like module; F: finger module

recent description of the crystal structure of plasminogen has confirmed and settled key structure-function relationships among its different constitutive modules.[12]

Structure of plasminogen activators

Both tPA and uPA have as plasminogen, a mosaic structure composed of several modules.[13] The characteristics of these molecules are reported in Table 1. The main function of the catalytic region of plasminogen activators is the conversion of plasminogen to plasmin by cleavage of the peptide bond Arg^{561}-Val^{562}. The tPA is secreted by endothelial cells as a single-chain protein with a very low index of zymogenicity.[2] This indicates that, exceptionally, the single-chain molecule is as active as the two-chain form generated after cleavage of the Arg^{275}-Ile^{276} of tPA by plasmin. However, the adsorption of these two forms of tPA onto the surface of fibrin via an interaction between the finger module and the D region of fibrin is a *sine qua non* for development of their activity.[1,14]

The uPA is released by leukocytes as a classical serine protease single-chain zymogen (sc-uPA), which must be transformed into a double-chain form (tc-uPA) in order to display its full protease activity.[5] The cleavage at the peptide bond Lys^{158}-Ile^{159} of sc-uPA is mainly made by plasmin. In contrast, thrombin wipes out the potential activity of scu-PA by cleaving it two residues upstream of the cleavage site of plasmin.[15] At the NH2-terminal position of the uPA is found the EGF module, which contains a sequence of interaction for its binding to the receptor uPAR. This receptor is itself anchored to the membrane via a glycosylphosphatidylinositol group that has a large transmembrane mobility. Beyond this function, the uPAR in concert with transmembrane glycoproteins activates several intracellular signaling pathways involved in cell migration and survival.[16]

Plasmin is formed on biological surfaces

Upon formation of a fibrin clot, plasminogen and tPA bind to its surface and acquire the molecular conformation necessary for the composition of an enzyme/substrate complex leading to *in situ* production of plasmin.[14] Plasmin formed remains bound to fibrin via its LBS and is thus engaged exclusively in a fibrinolytic function and protected from inhibition by α_2-antiplasmin. Indeed, the formation of a complex between the catalytic region of plasmin and the reactive site of the α_2-antiplasmin requires interaction with the LBS of kringle 1 of plasmin. Since plasmin remains adsorbed to fibrin it can not be inhibited.[17] Thus, to this surface reaction generating active plasmin can be opposed the reaction of inhibition by α_2-antiplasmin which takes place in the circulation. Lysis of a clot is therefore the result of a surface reaction with high specificity from the initial formation of plasmin to the acceleration and amplification phases of fibrinolysis.

Molecular interactions on biological surfaces

Plasminogen is immobilized onto lysine residues of fibrin via the LBS of kringles 1 and 4. The tPA, although having a kringle 2 module with an active LBS, binds to fibrin via its finger module. Inded, the affinity of the finger module for the D region of fibrin (Kd = 1 nM) is 1000 times greater than that of kringle 2 for the lysine residue (Kd = 1 µM). It is precisely this high affinity interaction with fibrin that allows expression of the activity of tPA. In the absence of fibrin tPA has only very limited ability to activate plasminogen. Conformational changes of plas-

minogen and tPA after adsorption onto their sites and their proximity on the surface of fibrin allow the composition of an enzyme/substrate complex resulting in the production of plasmin and the lysis of the fibrin polymer. In the vascular wall the generation of plasmin at the surface of macromolecules of the extracellular matrix follows the same principle: plasminogen is immobilized via its kringle domains onto fibronectin or laminin where it is activated by uPA released by inflammatory cells.[5]

On cell membranes, the molecular assembly occurs on receptor sites for plasminogen (α-enolase,[18] tetrameric complex annexin A2-S100A10,[19] histone H2B,[20] or the Plg-RKT[21]) where it is activated by uPA immobilized on its receptor uPAR. On certain cells such as endothelial cells, smooth muscle cells, or neurons, tPA is attached to transmembrane proteins that converts plasminogen to plasmin.[22,23] In contrast to uPA, no specific or unique receptor for tPA has been described.

Acceleration and amplification of fibrinolysis

Plasmin formed in situ can amplify the activation of plasminogen by generating new binding sites for plasminogen.[24] Indeed, the first molecules of plasmin generated at the fibrin surface, hydrolyze lysyl bridges and unveils carboxy-terminal (C-Lys) lysine residues that represent new plasminogen binding sites.[25] Plasminogen bound to C-Lys adopts an open conformation that is recognised and activated by tPA bound nearby or by uPA released from leukocytes. The uPA does not bind to fibrin but specifically recognizes the plasminogen bound to C-Lys sites.[4] The increased binding of plasminogen multiplies the number of molecules of plasmin formed by the activators and enhances the degradation of fibrin and clot lysis. It is the multiplication of the number of binding sites of plasminogen and the conformational changes thereof which are the major factors for the amplification fibrinolysis. The transformation of sc-uPA into double-chain uPA is a second acceleration factor in the formation of plasmin.[26]

Regulation

The plasminogen activation system is finely regulated by (1) serine protease inhibitors (serpins), (2) competitors of plasminogen, or (3) by proteolytic remotion of C-Lys binding sites.

(1) Serpins. Regulation by serpins directly affects plasmin (mainly α2-antiplasmin) or plasminogen activators (mainly PAI-1). In case of excess tPA or plasmin, plasma inhibitors with less restricted specificity can also act (α_2-macroglobulin, C1 esterase inhibitor).[27] In the central nervous system, other inhibitors of the plasminogen activation system such as neuroserpin and protease nexin-1 (PN-1) have been identifie.[28,29] PN-1 also inhibits plasmin and thrombin and recent data suggest that PN-1 stored in platelets could play an important role in the vascular system.[30] PAI-2 is produced by the syncytiotrophoblasts and monocytes. Its physiopathological role as an inhibitor of uPA and tPA remains an enigma, and seems to especially have intracellular functions.[31] It should be noted that in all cases the inhibition of activators or plasmin occurs mainly in the circulating or liquid phase and that in generally most players in the system of plasminogen activation are partially protected form their inhibitors when bound to their receptors.

(2) Competitors. Lipoprotein(a) or Lp(a) may exercise antifibrinolytic effect and many studies support the clinical relevance of this mechanism in cardiovascular disease.[32] The mechanism antifibrinolytic of this lipoprotein can be explained due to its particular structure, a component similar to the low density lipoprotein (LDL) and a glycoprotein, apo(a) structurally close to plasminogen but without enzymatic activity: a non-activatable copy of the catalytic region, a copy of the kringle 5 and a variable number of copies of the kringle 4 having a high affinity for fibrin.[33] Thus, competition between plasminogen and apo (a) for fibrin binding limits the amount of bound plasminogen, decreases the formation of plasmin and inhibits fibrinolysis.[34]

(3) Proteolytic remotion of C-Lys binding sites. The zymogen TAFI (thrombin-activated fibrinolysis inhibitor; procarboxypeptidase U) can be activated by thrombin or plasmin in TAFIa. This exopeptidase cleaves the Lys-C residues of proteins, thereby limiting the binding of plasminogen to the activation surfaces.[35] The in vitro activity of TAFIa is well established[36] but it does not seem to play a role in physiological fibrinolysis in vivo (mouse mode).[37] The important number of studies supporting the clinical interest for this potential regulator of fibrinolysis, has so far provided only associational results.[38]

Finally, in contrast to the mechanism of regulation of plasminogen receptors by TAFIa, agents that block the LBS of kringle modules can be used as therapeutic inhibitors of the binding of plasminogen. These are lysine

analogues such as e-ACA and TXA mentioned above. These compounds interact with the LBS of kringle and thus block competitively the binding of plasminogen to fibrin or cells. Their clinical use as an anti-fibrinolytic and anti-haemorrhagic has recently been discussed in several clinico-surgical situations and in a large multicenter study.[39,40]

Functions of the plasminogen activation system

We distinguish the functions of the plasminogen activation system (fibrinolysis and pericellular proteolysis) depending on the nature of the surface on which the reaction takes place.

- Fibrinolysis: cleavage by plasmin of arginyl and lysyl bridges of fibrin leading to its dissolution and release of degradation products. D-dimer fragments found in the circulation reflect both formation of clot and its dissolution by plasmin.[1] Efficient fibrinolysis allows recanalization of the occluded vessel. The use of thrombolytic agents for the treatment of ischemic stroke or coronary events is modeled on this physiological model.
- Pericellular proteolysis occurs when the formation of plasmin occurs at the surface of cell membranes or the extracellular matrix.[22, 23, 26] At the cellular level, plasmin activates transmembrane receptors (PAR, protease-activated receptors 1 and 4), induces intracellular signalling[41] and a phenotypic response characterized initially by membrane vesiculation.[42] The released microvesicles bear plasminogen activators synthesized by the parent cell.[42] The plasmin formed in situ induces directly or via the activation of pro-metalloproteases (MMP-3, 9 and 12) the proteolysis of matrix proteins: fibronectin, laminin or vitronectin.[43] This proteolysis induces changes in cell adhesion leading to different physiological phenomena (cellular remodelling, angiogenesis, cell migration).[36] The plasmin formed in excess or resulting from lack of regulators (inhibitors) produces a degradation in extenso of the extracellular matrix. This process can result in the loss of cell adhesion and death by apoptosis as observed in some pathological situations (cell death, weakening/rupture of the atherosclerotic plaque, aneurysm).[44-46] This process of apoptosis induced by cell detachment may be thwarted by inhibitors such as PAI-1 and protease nexin-1.[44-47] It is important to differentiate these stages of cell activation and apoptosis to assess the effects of mediators, inhibitors and therapeutic agents.

Fibrinolysis, peculiarities: platelets, microvesicles

The classical pathway of plasminogen activation presented above requires the co-assembly of plasminogen and its activator (uPA or tPA) on the same surface in order to trigger the fibrinolytic or proteolytic process. Moving surfaces such as platelets and microvesicles require special conditions for the production of plasmin.

Mechanism of plasminogen binding to platelets

Like other cell membranes, platelets can adsorb plasminogen on their surface via C-Lys residues-dependent interactions which number is multiplied by 5 on activated platelets (specific binding, saturable and reversible).[6] This binding is made via the GPIIb / IIIa (αIIb β3) and fibrinogen (fibrin) of platelets activated with thrombin. [48, 49] Plasminogen thus bound adopts an open conformation more easily activated by uPA. Platelets may thus contribute to increase the concentration of plasminogen and potentially plasmin within the clot despite their procoagulant activity.

Microvesicles and the plasminogen activation system

Microvesicles are membrane vesicles released by activated cells or cells in apoptosis.[50] Of size between 0.1 and 1μm they have exposed phosphatidylserine on their surface, contain no DNA fragments and must not be confused with apoptotic bodies or exosomes (Figure 2).[51] The formation and release of microvesicles is the result of an extracellular stimulus (physical, chemical or biological) that leads to a massive influx of calcium into the cell. The increase in intracellular calcium alters the activity of phospholipids transporters and stimulates calpains resulting in phosphatidylserine externalization, changes in the integrity of the cytoskeleton and cell contraction leading to budding of microvesicles from the cell membrane.[52] Many pathological conditions such as cardiovascular disease, diabetes, cancer and inflammatory diseases have been associated with an increase in the number of microvesicles.[53,54]

These microvesicles carry on their surface and in their cytoplasm proteins of the parent cell. In addition to cell type specific clusters of differentiation (CD), various biomolecules including tissue factor (TF) and inflamma-

tory cytokines can be vectorized by microvesicles.[55,56] In 2007, it was shown that microvesicles released from TNF-stimulated HMEC-1 cells (a cell line that synthesizes uPA and its receptor uPAR) with were able to generate plasmin.[8] Indeed, this type of microvesicles bears at their surface uPA / uPAR complexes and uPAR available sites capable of binding exogenous uPA (Figure 2).

The binding of plasminogen to the surface of the microvesicles also involves residues C-Lys. A selective antibody directed against the α-enolase confirmed that this receptor of plasminogen was involved in the binding of plasminogen to the surface of endothelial microvesicles. Recently it has been also shown that the intracellular protein, histone H2B, was involved in the binding of plasminogen to the surface of microvesicles.[20] The histone H2B is localized to the cell membrane via an interaction with phosphatidylserine exposed in the outer leaflet of the membrane.[57] Thus, procoagulant phospholipid would also increase the number of plasminogen binding sites and promote fibrinolysis.

We confirmed the presence of fibrinolytic microvesicles in the circulation.[58] [è!] These microvesicles have functional characteristics similar to those previously described and bear plasminogen activators synthesized by the parental cell (leukocytes: uPA, endothelial cells: tPA). These findings underscore the pathophysiological relevance that such microvesicles could have in vivo.

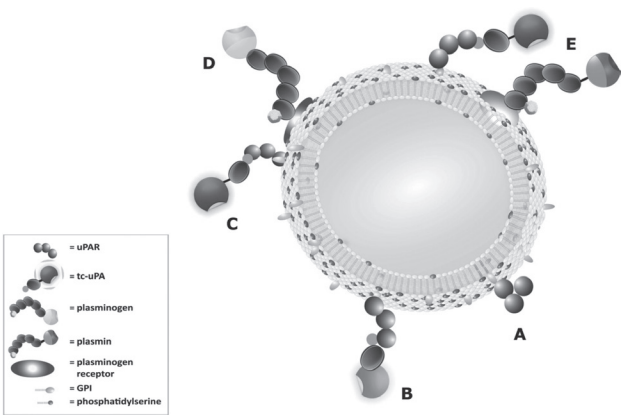

Figure 2. Plasminogen activation on a microvesicle.
Representation of a fibrinolytic microvesicle bearing on its surface (A) the uPAR receptor anchored to the membrane by the glycosylphosphatidylinositol (GPI) group. (B) conformational change of uPAR after binding of sc-uPA. (C) sc-uPA is converted in situ into tc-uPA. (D) plasminogen bound to its receptor adopts the open form. (E) plasmin generated on the membrane by tc-uPA.

A new pathway of plasminogen activation: fibrinolytic cross-talk

Plasminogen adsorbed to fibrin or to the cell membrane adopts the open molecular conformation whose cleavage site is easily accessible to activators located nearby on the same surface. This is the case of the plasminogen-fibrin-tPA ternary complex or of the plasminogen-cell membrane-uPAR/uPA assembly. However, it was found that uPA in solution was an efficient activator of Lys-plasminogen and of Glu-plasminogen complexed to e-ACA. These observations allowed us to hypothesize the existence of an interaction involving two surfaces, one bears plasminogen and the other an activator of plasminogen.[9] It was possible to show that plasminogen carried by platelets can be recognized by cells or microvesicles bearing the complex uPA/uPAR. In a similar fashion the plasminogen bound to fibrin or matrix proteins can be activated to plasmin by uPA/uPAR borne by leukocyte microvesicles. This new mechanism of activation of plasminogen that we have called fibrinolytic cross-talk (Figure 3) is characteristic of uPA and is therefore not sensitive to microvesicles bearing tPA. This specificity could be explained by structural arrangements imposed by the different modules of tPA (finger-EGF-K1-K2-SP) and uPA (EGF-K1-SP) (Table 1). This activation reaction has therefore all the characteristics of a specific and saturable reaction whose efficiency depends on the number of active microvesicles acting on platelets or fibrin. This new activation pathway may have a role physiologically relevant. Indeed, the activation of plasminogen on the surface of platelets by microvesicular uPA generates two times more plasmin that uPA in solution. Recent studies by independent laboratories have confirmed our hypothesis and results.[59,61] Two studies have reported the activation of plasminogen bound to fibrin by uPA borne by leukocytes[59,61] while the third study is focused on the activation of sc-uPA by plasmin formed on the platelet surface.[60]

Relevance of the fibrinolytic cross-talk mechanism

This novel mechanism of activation of plasminogen to plasmin at the surface of platelets, the extracellular matrix and fibrin by uPA raises the question of its involvement in different pathophysiological situations:

Fibrinolysis

The binding of plasminogen to platelets during clot formation and the presence of microvesicles bearing uPA

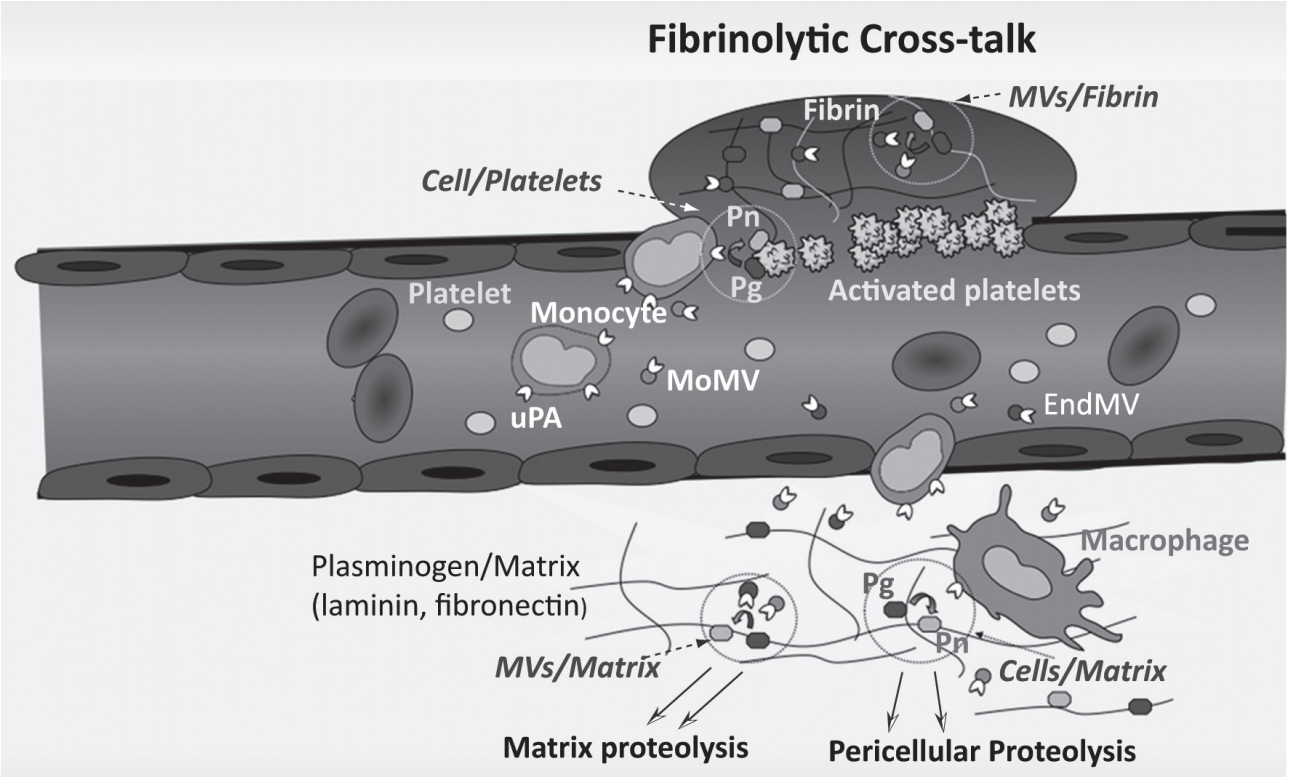

Figure 3. Fibrinolytic cross-talk (Blood 2010, 115(10) cover illustration).
In inflammatory processes of the vascular wall, fibrinolysis and proteolysis may be induced via a cross-talk between monocytes or cellular microvesicles bearing uPA and platelet-, fibrin-, or extracellular matrix–bound plasminogen. This mechanism of plasmin formation bypasses the requirement for co-assembly of plasminogen and uPA on the same surface. See the article by Dejouvencel et al on page 2048.
The fibrinolytic cross-talk refers to the interaction that is established between two biological surfaces, one carrying plasminogen, the other uPA. The surfaces carrying plasminogen (Pg) are represented by the platelet membrane, fibrin or extracellular matrix proteins. The surfaces bearing uPA are represented by microvesicles (MVs) issued from leukocytes (MoMV). The microvesicles are moving surfaces, this inter-surface activation system allows efficient generation of plasmin (Pn) on the surface of fibrin or platelets forming the clot and on proteins of the extracellular matrix. Endothelial MVs (EndMV) bear tPA.

would lead to the formation of plasmin and allow the re-canalisation of an occluded vessel. Indeed, a recent study suggests that the activation of plasminogen bound to fibrin by leukocytes bearing uPA plays a role in endogenous fibrinolysis.[61]

Cell migration and angiogenesis

Aside from its fibrinolytic function, plasmin formation by the uPA/uPAR system is involved in tissue remodelling via the activation of proMMP and plays a critical role in cell migration and angiogenesis.[16] Indeed, vascular regeneration involves both angiogenesis and vasculogenesis-dependent endothelial progenitor cells. The ability of endothelial microvesicles to generate plasmin influences and modulates the repair process of endothelial progenitor cells. A small amount of microvesicles bearing an active plasminogen activation system, promotes cell migration and angiogenesis whereas at high concentrations the excess plasmin leads to matrix degradation, decreased cell adhesiveness and finally apoptosis.[8,35]

Dissemination of cancer cells

The spread of cancer cells is a consequence of matrix degradation and loss of cell adhesion. High amounts of uPA/uPAR were associated with advanced metastatic cancers.[62] It is interesting to note that the described fibrinolytic/proteolytic cross-talk mechanism is only possible in the presence of an activator of the uPA-type. This activa-

tor is involved in tumour progression and was found on microvesicles emitted by cancerous cells. In addition, microvesicles released by platelets may promote metastasis and promote angiogenesis.[63]

Conclusion and potential applications

The structure and function of molecules of the plasminogen activation system including the recent description of the crystal structure of plasminogen,[12] and their 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 for the haematologist and the vascular biologist. The circulating concentration of plasminogen activators is extremely low compared to active concentrations required at the site of injury in the microcirculation. Moreover, the plasminogen activators circulate as an inactive complex with PAI-1 and only the forms located on the cell membrane (uPA, tPA) or fibrin (tPA) are active. Furthermore, since all measurements performed in plasma do not take into account the contribution of cellular activators, it is therefore impossible to quantify a lack of tPA or uPA activity that may be the cause of a fibrinolytic default. The recent discovery of cellular fibrinolytic microvesicles and a new mechanism for the formation of plasmin, the fibrinolytic cross-talk, opened up new perspectives.[8] These microvesicles would act within the clot, thus explaining the lack of systemic fibrinolysis as demonstrated in vivo in a mouse model of fibrinolytic platelets.[64] We suggest that the fibrinolytic activity of endothelial and leukocyte microvesicles compensates locally the activity of procoagulant microvesicles. Is this phenomenon that explains the spontaneous re-canalization observed in 10-20% of patients with acute occlusion of the coronary arteries?[65,66] Accordingly, the functional balance between these two types of microvesicles would result in a physiological haemostatic response, while the lack of fibrinolytic microvesicles may promote the formation of a thrombus. The existence of a haemorrhagic syndrome (Quebec platelet disorder) caused by profibrinolytic platelets having an abnormal expression of uPA is consistent with this hypothesis.[67] Using a mouse model of this autosomal dominant disease it was possible to demonstrate that these animals are resistant to arterial thrombosis and that transfusion of these platelets to control mice prevent the formation of occlusive arterial thrombi.[67]

In this context, the presence of plasminogen and its activator uPA or tPA on moving surfaces (respectively platelets and microvesicles) and the identification of the fibrinolytic cross-talk mechanism suggests the possibility of using these materials as vectors of fibrinolysis and pericellular proteolysis.

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