Plasma kallikrein: the bradykinin-producing enzyme

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Summary
Plasma prekallikrein is the liver-derived precursor of the trypsin-like serine protease plasma kallikrein (PK) and circulates in plasma bound to high molecular weight kininogen. The zymogen is converted to PK by activated factor XII. PK drives multiple proteolytic reaction cascades in the cardiovascular system such as the intrinsic pathway of coagulation, the kallikrein-kinin system, the fibrinolytic system, the renin-angiotensin system and the alternative complement pathway. Here, we review the biochemistry and cell biology of PK and focus on recent in vivo studies that have established important functions of the protease in procoagulant and proinflammatory disease states. Targeting PK offers novel strategies not previously appreciated to interfere with thrombosis and vascular inflammation in a broad variety of diseases.

Protein chemistry
Plasma prekallikrein (PPK) is a glycoprotein that is predominantly synthesised in the liver. Low levels of PPK mRNA are also found in the epithelial cells of the kidney, adrenal gland and placenta. PPK is secreted into the circulation as a single chain zymogen that has an apparent molecular weight of approximately 85-88 kDa and migrates on SDS-PAGE as a doublet in the presence or absence of reducing agents. The calculated molecular weight for the protein product of the mRNA is 79.5 kDa, suggesting that the doublet likely reflects two differentially glycosylated forms (3). PPK has a concentration of ~580 nM (50 µg/ml) in plasma of healthy individuals and circulates mostly as a complex with high-molecular-weight kininogen (HK) (75-90%). The mature PPK form, which is devoid of the signal peptide, is stabilised by an arrangement of 18 disulfide bridges. PPK is further modified by five N-linked carbohydrate chains and three on the light chain. The glycoprotein migrates with the γ-fraction of human plasma on serum protein electrophoresis and has an apparent isoelectric point of 8.5-9.0, a sedimentation coefficient of 5.2 S and an extinction coefficient of 11.7 at 280 nm (2, 3).

PK mRNA codes for a single chain polypeptide of 638 amino acid residues including an N-terminal signal peptide sequence of 19 residues (pro-prekallikrein). The PPK protein sequence of 619 residues is composed of five domains. The N-terminal portion consists of four tandem repeats of 84 to 85 residues each, called ‘apple’ domains (designated A1 to A4). The four apple domains are followed by 248 residues comprising the catalytic domain of the protein (amino acids 372 - 619) (3). The major physiological activator of PPK, activated factor XII (FXIIa), cleaves a single peptide bond (Arg371-Ile372) to generate a two-chain molecule with a heavy chain of 371 and a light chain of 248 residues held together by a disulfide bridge between Cys364 on the A4 domain and Cys484 on the serine protease domain (4) (Figure 1). The two-chain protein is the active form of plasma kallikrein called α-PKa. Cleavage at Arg371-Ile372 results in a conformational rearrangement of the light chain and generates the catalytic active serine protease. Upon prolonged incubation (>72 hours), α-PKa auto-catalytically converts to β-PKa through an additional cleavage at the Lys140-Ala141 bond within domain A2 (5) (Figure 2). The physiological importance of this reaction is still unclear. PK shares high homology with blood coagulation factor XI (FXI) and the proteases are 58% identical on the amino acid level, yet they differ in their gross structures. PK is a monomer whereas FXI is a homodimer. In contrast to PK, FXI has a single free Cys residue in A4 (position 321) that forms an interchain disulfide bond with the same residue in a second FXI polypeptide. Both PK and FXI form tight 1:1 complexes with HK with KDs of 12 nM and 18 nM, respectively. In the case of PK, the apple domain A2 has been identified as the major binding segment for the HK domain D6H, with domains A1 and A4 contributing to high-affinity binding (5-7). PK is anchored to cell surfaces by binding to its substrate HK. PPK and PK bind to HK with similar affinity, indicating that proteolytic activation of PK does not interfere with the stability of the PPK/HK complex (5, 7, 8).
Gene structure and evolution

The human PK gene is approximately 22 kbp in length, and maps to chromosome 4q34-q35. The PK gene consists of 15 exons and 14 introns. Exon 1 (E-1) codes for the 5’ untranslated region of the mRNA and E-2 encodes the signal peptide sequence. Exons 3 to 10 code for the 4 homologous apple domains (2 exons per apple domain). The exon pair’s coding for the individual apple domains share extensive sequence homology suggesting that they arose from a common ancestor by two successive duplication events. Exons 9 to 15 encode the catalytic domain of PK containing the catalytic triad of His415, Asp464 and Ser559 (9). The only other known member of the PK gene family is the FXI gene, which maps to the same chromosomal locus and has the identical exon-intron structure. This suggests that PK and FXI genes most likely arose by a gene duplication event (10). Apple domains are members of the PAN (Plasminogen, Apple, Nematode) domain family, with homology to the N-terminal domains of plasminogen and hepatocyte growth factor (HGF) (11). HGF is found in the genomes of all vertebrates, and it is likely that both FXI and PK apple domains descend from an HGF-like protein. A gene coding for a trypsin-like protease with four PAN domains is found in the frog (Xenopus), green anole lizard (Anolis carolinensis), and chicken (Gallus gallus) (12).

FXI and PK are distinct genes present on chromosome 4 in humans. A single PK/FXI predecessor gene is found in an egg-laying mammal the platypus (Ornithorhynchus anatinus). Distinct genes for FXI and PK are however, present in the genome of the opposum (Monodelphis domestica), a marsupial, as well as in placental (eutherian) mammals (12). The duplication of the gene for the PK/FXI predecessor that resulted in individual FXI and PK genes, likely occurred during mammalian evolution, probably after monotremes diverged from the lineage leading to marsupials and eutherian mammals. The genes for FXI and PK make their first appearances together in amphibians (they are not found in fish) (12). Thus PK is a “modern” protein that has evolved lately during evolution and is found in mammals only.

Plasma kallikrein deficiency (fletcher trait)

PK deficiency is rare and only 80 cases of congenital severe PK deficiency have been reported (13). Inherited deficiency in PK is not accompanied by any obvious specific symptom or pathological condition. It is likely that most cases are undetected or, if detected, remain unreported. Hathaway et al. described the first family with PK deficiency back in 1965 (89). Affected family members presented with prolonged plasma-clotting times that were corrected in vitro upon prolonged incubation with glass. The trait is autosomal-recessive, and the missing plasma component, named “Fletcher” factor after the index cases, was subsequently shown to be PK. Although severe PK deficiency is a rare condition, many mild cases may be missed because mild PK deficiency is clinically asymptomatic (only 2-5% of normal PK activity is sufficient to bring most activated thromboplastin time (aPTT) assays into the normal range) and because some aPTT reagents (e.g. elagic acid) are not sensitive enough to detect low levels (14-17).

There are two principal types of hereditary PK deficiency in patients. Type I (cross-reacting material [CRM]-negative forms), the most prevalent type, which is associated with a decrease in or absence of PK activity and antigen, and type II (CRM-positive forms), in which PK enzymatic activity is low, but antigen is variably present. The exact prevalence in the general population is unknown, and the majority of reported patients with PK defects rep-
resent the type I deficiency. Approximately 10–15% of cases show a type II pattern. In addition, there are patients that are considered CRM-reduced. These patients have antigen levels of 10 or 35% of normal, and carrying PK Zürich mutation or PK Long-Beach mutation, respectively. (13).

Activation of plasma prekallikrein

PK is a member of the plasma contact system, which encompasses FXI, FXII and HK. The name “contact system” reflects the fact that the proteins are activated by FXII binding (“contact”) to negatively charged surfaces (18, 19). Contact of FXII to surfaces induces a conformational change in the zymogen resulting in autoactivation of the molecule and increased susceptibility of surface bound FXII to be processed by PK. Initially, a small amount of FXIIa is formed that converts PPK to the active protease PK. PK then reciprocally activates new FXII zymogens. The feedback loop amplifies FXII activation and initiates the intrinsic pathway of coagulation via FXIIa-mediated activation of FXI (20, 21). Additionally, once PPK is converted to the active form, α-PKα cleaves HK to release the potent inflammatory mediator bradykinin (BK, Kallikrein-kinin system) (2) (▶ Figure 3). A number of chromogenic substrates including H-D-prolyl-L-phenylalanine-L-arginine-p-nitroanilide and N-benzoyl-L-prolyl-L-phenylalanine-L-arginine-p-nitroanilide are available to access the proteolytic activity of PK. Typically the cleavage site of these synthetic peptide derivatives mimics the activated factor XII (FXIIa) cleaves PK at a single site to generate a three-chain form with two intercatenar disulfide bridges (β-PKα). Autocatalytic processing yields the three-chain form with two intercatenar disulfide bridges (β-PKα). Asterisks indicate the relative positions of the residues of the catalytic triad (His415, Asp464 and Ser559).

C1INH forms a non-hydrolysable bond with the active site serine of α-PKα, blocking the proteolytic activity of the enzyme. In addition, α2-macroglobulin (α2M), antithrombin III (23), plasminogen activator inhibitor-1 (24), and protein C inhibitor interfere with PK activity in plasma and on cell surfaces (25) (see chapter Hereditary angioedema below).

The contact system components bind to vascular endothelium (26, 27), platelets (28), and neutrophils (29), with HK serving as a docking site for PK (27). HK binds to cells via heparan and chondroitin sulfate glycosaminoglycans (GAG) of cell surface proteoglycans (30, 31). FXII may also bind to GAGs, facilitating contact system protein assembly on endothelial cells. GAGs do not only assemble contact system proteins on cellular surfaces, the polysaccharides also critically regulate BK formation. HK binding to GAGs interferes with contact system-driven BK generation, indicating that HK needs to dissociate from GAGs to allow for efficient PK-mediated cleavage (32). In lysed cell extracts HK binds to the uPA receptor (CD87), cytokertin 1 (a cytoskeleton component) and “globular Clq receptor” (gClqR/p33; (33, 34) that has a mitochondrial targeting sequence. Overexpression of gClqR/p33 does not increase HK binding to transfected cells. In contrast overexpression of heparan sulfates largely intensifies HK cell binding (30).

Contact-activated FXIIa in turn activates PPK. Multiple non-physiological FXII activators including glass, metals and certain polymers have been identified (37). Naturally occurring FXII contact activators include collagen, cholesterol sulfate, sulfatides, acid phospholipids, fatty acids, and several charged carbohydrates (38). Recently, in vivo FXII contact activators including polyphosphates (polyP), mast cell released heparin and misfolded proteins have been identified (39-41). PolyP is an inorganic non-branched polymer of ortho-phosphate residues that is released from activated platelets and initiates FXIIa autoactivation inducing PK-mediated HK cleavage (42). Mast cells initiate PK-mediated BK formation by another mechanism (40). IgE/allergen-activated
mast cells release the polysaccharide heparin that efficiently induced FXII autoactivation in plasma and in mouse models in vivo (40). Heparin is a linear, unbranched, and highly sulfated polymer consisting of repeated disaccharide units that is exclusively stored in mast cell and basophil secretory granules. Heparin-initiated FXII activation selectively initiates PK activation culminating in BK formation. In contrast to polyP heparin does not initiate the FXIIa-driven intrinsic pathway of coagulation (40). PolyP is also a component of mast cell granules, suggesting that polyP may contribute to mast cell-stimulated BK formation (43). Misfolded protein aggregates, such as those found in plasma of patients with amyloidosis, have been shown to promote FXII and PK activation.

Figure 3: Plasma kallikrein-driven signalling cascades (A–E) and functions in disease states (F–I). A) Plasma prekallikrein (PPK) is converted to plasma kallikrein (PK) by FXIIa (kallikrein-kinin system), PK cleaves high-molecular-weight kinogen (HK) and releases the inflammatory mediator bradykinin (BK). BK is a peptide hormone and binding to its receptors triggers vasodilation and increases permeability causing vascular leakage. B) PK also reciprocally activates FXII zymogens, thereby initiating the intrinsic pathway of coagulation via FXIIa-mediated activation of FXI culminating in clot formation. C) PK stimulates fibrinolysis by converting plasminogen to plasmin or by activating pro-urokinase type plasminogen activator (pro-uPA) to uPA. Plasmin could also activate pro-uPA, in turn; active uPA activates plasminogen to plasmin, which results in clot lysis. D) In the alternative complement pathway, PK can substitute for factor D to activate the alternative pathway C3 convertase of human complement. Factor D cleaves factor B, which is part of the active C3 convertase C3bBb subsequently activating host-defense reactions. E) PK also acts in the renin-angiotensin system (RAS), where it converts prorenin to renin involved in blood pressure regulation. F) Hereditary angioedema is a rare disorder associated with life-threatening swelling attacks that develop in individuals deficient in C1INH (HAE type I), or individuals with a dysfunctional C1INH protein (HAE type II). C1INH is the major inhibitor of PK and regulates generation of the inflammatory mediator BK released from HK. Excessive BK formation results in oedema formation. G) Patients with diabetic retinopathy present with elevated carbonic anhydrases I/II activities. Extracellular carbonic anhydrases enhance the intraocular pH and thus activate PK that in turn releases BK, which increases retinal vascular permeability, leading to retinal oedema and vision impairment. H) Intracerebral haemorrhage (ICH) is bleeding into the brain where PK inhibits collagen-induced platelet activation in hypoglycaemic states. Black filled circles represent collagen. I) The role of PK in thrombosis and haemostasis has been shown in PK-deficient mice. Thrombosis in arterial and venous vascular beds is largely defective in PK null mice whereas the mice have a normal haemostatic capacity.
FXIIa independent modes of PPK activation involving heat shock protein 90 (Hsp90, an chaperon) and prolylcarboxypeptidase (PRCP, an exopeptidase (46)), have been described in ex vivo and cell culture systems, however there in vivo relevance remain topic of future research (47, 48).

Plasma kallikrein-driven signalling cascades

Kallikrein-kinin system

PK cleaves HK to release the inflammatory mediator BK (2) (Figure 3). BK is a peptide hormone and acts through stimulation of G-protein coupled B1 receptors (B1R) or B2 receptors (B2R). BK signalling is mostly mediated by binding to B2R, which have high affinity for BK (8-12 nm) and are constitutively expressed in multiple tissues. B1R are expressed in very low copy numbers under "normal" conditions but expression may be rapidly increased in response to tissue injury, inflammation, or other pathophysiological events in an interleukin (IL)-1β and tumour necrosis factor (TNF)α-dependent manner (49-52). BK binding to both B1R and B2R increases intracellular calcium ([Ca2+]i) in smooth muscle and endothelial cells. [Ca2+]i, in turn can activate multiple signalling cascades, including the phospholipase A2 pathway, which releases arachidonic acid that is converted to prostacyclines (e.g. PGI2) in a cyclooxygenase-dependent manner. BK also triggers protein kinase C (PKC) activity (53, 54) resulting in VASP-mediated disassembly of cortical cytoskeletons (55) and VE-cadherin junctions (56). Increase in [Ca2+]i is a potent stimulus of endothelial nitric-oxide synthase (eNOS) resulting in nitric oxide (NO)-driven protein kinase G activity and VASP phosphorylation (57, 58). BK signalling increases Rac1-driven vascular leakage (59). All these pathways have a role in BK-driven vasodilation and increased vascular permeability (60). The principal enzymes for bradykinin degradation are carboxypeptidases M and N (kininase 1), angiotensin converting enzyme (kininase 2), and neutral endopeptidase (61).

Intrinsic pathway of coagulation

PK also reciprocally activates FXII zymogens. This feedback loop amplifies FXII activation that initiates the intrinsic pathway of coagulation via FXIIa-mediated activation of FXI (20, 21). FXIIa cleaves its substrate FXI to form active FXIa, which in turn promotes coagulation via Ca2+-dependent activation of FIX. The extrinsic and intrinsic pathways converge on FX. FXa in complex with the cofactor FVa converts prothrombin into thrombin (Figure 3). Thrombin activates multiple pathways in the vascular system (62). In addition to cleaving fibrinogen to form fibrin, thrombin can amplify its own generation by activating FXI (63).

Fibrinolysis

Fibrinolysis is the process that redissolves the fibrin network, mainly by action of the protease plasmin. Plasmin is produced by conversion of its precursor plasminogen and that reaction is activated by tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA). In vitro, using a buffer system PK converts plasminogen to plasmin (64). In plasma, PK has the capacity to activate plasminogen directly; however, the in vivo role of this step remains to be demonstrated. PK mostly promote fibrinolysis indirectly by activating the proenzyme form of uPA (pro-uPA) to its enzymatically active form, uPA (Figure 3). Addition of HK does not affect plasminogen activating activity of purified PK (65).

Renin-angiotensin system (RAS)

PK activates the blood pressure-regulating renin-angiotensin system by converting prorenin to renin (66) (Figure 3), which converts angiotensinogen to angiotensin I. Angiotensin-converting enzyme (ACE) converts inactive angiotensin I to angiotensin II and functions as a kainase by degrading BK (67). Thus PK regulates BK signalling on two independent levels. The protease forms BK by conversion of HK and amplifies the BK degrading renin-angiotensin pathway.

Alternative complement pathway

The alternative complement pathway is initiated by complex polysaccharides as well as immune complexes of the IgA class, which interact with factors B, D, C3, and properdin to yield a stabilised C3 convertase consisting of C3bBb (68). PK can replace factor D for activation of the alternative pathway C3 convertase of human complement. Factor D cleaves factor B, which is part of the active C3 convertase C3bBb (Figure 3). The ability of PK to cleave factor B is dependent on magnesium ions and available C3b. However, factor D is about 10-fold more effective as compared to PK on a molar basis, for activating the alternative pathway C3 convertase. The importance of PK for complement system host-defense reactions in vivo is not yet clear; however, some individuals with severe deficiency in contact factors have been reported to suffer from increased susceptibility to bacterial infections (69).

Plasma kallikrein functions in disease states

Hereditary angioedema

Hereditary angioedema (HAE) is a rare autosomal dominant disorder associated with life-threatening swelling episodes that develop in individuals deficient in C1INH (HAE type I), or individuals with a dysfunctional C1INH protein (HAE type II). C1INH is the major inhibitor of the classical complement pathway proteases C1r and C1s and regulates generation of the inflammatory mediator BK by the plasma contact system (PK, FXII) (61). Deficiency in or a non-functional C1INH facilitates excessive activation of the
complement and contact system cascades and BK-mediated oedema formation in HAE type I and II patients (70, 71). Other inhibitors of α-PKα are α2-macroglobulin, antitrypsin and antithrombin-III (ATIII). However, they cannot compensate for the complete deficiency in C1INH. In addition to these two classical HAE types a third variant exists that almost exclusively affects women. HAE type III patients have normal biochemical C1INH function and plasma C1INH concentration, but also suffer from oedema, due to a single point mutation in FXII (72). The mutation is outside the enzymatic FXII domain and the underlying mechanisms how this specific mutation could induce swelling episodes in HAE type III patients are not understood. Gene-deficient mouse models have shown that oedema formation in C1INH-dependent forms of HAE is due to aberrant B2R signalling (73). Genetic ablation of C1INH expression results in excessive BK production, which increases vascular permeability in humans (74) and mice (40) (Figure 3). BK plasma levels are elevated in acute swelling attacks, whereas in the interval BK levels in HAE patients are similar to healthy controls (remission phase) (75). Accordingly, acute swelling attacks in HAE patients respond to infusion of plasma purified or recombinant C1INH, B2R antagonists, and PK inhibitors that block the BK-producing pathway on various levels (76).

Diabetic retinopathy

Diabetic retinopathy is the advanced stage of a condition that affects about 12% of individuals with diabetes type II. A comprehensive proteomic analysis in individuals who had proliferative retinopathy versus individuals with diabetes but no retinopathy or individuals who were not diabetic revealed that carbonic anhydrases I/II were elevated >8-fold (77, 78). Extracellular carbonic anhydrase is a major component of the vitreous gel in individuals with advanced disease. The enzyme directly increases the vascular permeability in the retina, leading to retinal oedema and vision impairment. The suggested mechanism is that carbonic anhydrases I/II increase the intraocular pH and this activates PPK to PK, resulting in enhanced BK-mediated vascular permeability (Figure 3). The vascular permeabilising effect of carbonic anhydrase was inhibited by C1INH (which inhibits FXII and PK), inhibitors of B2R, anti-PK antibodies and control of vitreous pH (77).

Intracerebral haemorrhage

Intracerebral haemorrhage (ICH) occurs when a weakened blood vessel ruptures resulting in bleeding into the surrounding brain, as is occasionally seen in people with hypertension. The severe vascular injury triggers a rapid haemostatic response that involves adhesion and aggregation of platelets and the activation of the coagulation system. This cascade generates thrombin, which further activates platelets and converts plasma fibrinogen into fibrin that stabilises the clot (79). Hyperglycaemia promotes ICH through PK-mediated inhibition of the platelet-vessel wall interaction. A landmark article by Liu et al. showed that PK exerts potent antihaemostatic function in diabetic rodent models and that this effect is greatly enhanced in hyperglycaemic blood (80). Hyperglycaemia may induce binding of PK to exposed collagens at the site of vascular injury by yet-unidentified mechanisms. Adhesion of PK to collagens blocks binding sites for platelet glycoprotein VI receptors (GPVI, the activating collagen receptor on platelets) and inhibits platelet aggregation on collagen fibres at the injury site, leading to sustained bleeding and haematoma expansion (Figure 3). These findings suggest targeting PK for prevention of brain haematoma in hyperglycaemic patients (80). Thus, inhibition of PK may not only prevent thrombotic events in ischaemic stroke (81), but also simultaneously lower the risk of ICH in hyperglycaemic animals. Further studies are required to test this intriguing hypothesis in animals and, if confirmed, also in patients with ICH.

Role in thrombosis and haemostasis

Recently, Bird et al. generated and phenotyped PK deficient mice in various models of thrombosis and haemostasis. Severe deficiency of PK abolishes experimental thrombosis in arterial and venous vascular beds. Despite the thromboprotective effects, PK deficiency has minor effects on the haemostatic capacity (Figure 3) (82). Consistent with a critical role of contact system proteins in thrombosis but not in haemostasis, FXII- and HK-deficient mice also have an antithrombotic phenotype and do not bleed excessively (83, 84). These results demonstrate that contact system-driven fibrin formation is specifically important for pathologic thrombus formation but has no function for fibrin formation during “normal” haemostasis. This raises the possibility that targeting PK may offer a strategy for prevention or treatment of thrombosis that is not associated with the high rate of haemorrhage that accompanies currently used anticoagulants (85).

Inhibitors of plasma kallikrein

Patients with partial and severe PK deficiency display no pathological bleeding symptoms or other reported complications; however, PK functions in multiple diseases, detailed above. Taken together, inhibiting PK could be an effective treatment strategy without major adverse side effects.

C1 esterase inhibitor

C1INH is the major endogenous inhibitor of PK and is used during replacement therapy in patients with type I and II HAE. Intravenous infusion of recombinant and plasma derived C1INH concentrate shortens the extent and duration of acute swelling attacks. Additionally when used for prophylaxis the concentrate reduced the frequency of acute attacks (86).

Aprotinin

Aprotinin, bovine pancreatic trypsin inhibitor, is a small protein, which inhibits several serine proteases including PK. The drug Aprotinin (Trasylol®) is an antifibrinolytic agent used primarily in
complex and/or redo cardiac surgery as an adjunct to decrease postoperative bleeding and to reduce organ damage caused by hypotension. However in 2008, the results of the Blood Conservation Using Antifibrinolytics in a Randomised Trial (BART) challenged the safety of Aprotinin. Although data were not reproduced by independent trials and were only slightly above the statistical significance level the drug was withdrawn from the market within some months, because of a higher rate of death in patients receiving Aprotinin compared to other antifibrinolytic agents (87).

**Antisense oligonucleotides**

A new and exciting method to inhibit PK activity is based on anti-sense technology (14). Selective antisense oligonucleotides (ASO) mediate knockdown of PK expression for several weeks in mice. However, the drawback with this method is that the ASOs need to be repeatedly applied by subcutaneous injections twice per week for three weeks to achieve sufficient knockdown of PK expression. Although effects on BK formation were not analysed, it was reported that ASO treatment inhibits thrombosis in venous and arterial thrombosis models, without affecting haemostasis (14). Furthermore, ASO-mediated PK depression reduces FXII activation in mice, consistent with PK contributing to thrombus formation through activation of FXII. PK ASOs are highly specific for their target PK and do not affect mRNA expression of coagulation factor II (prothrombin), VII, XI and XII (14), suggesting that PK ASOs may be a promising preventive therapeutic strategy in HAE patients.

**Conclusion**

Here, we review PK structure, functions, activation, roles in experimental animal models and importance for human disease states. PK drives various signalling pathways including the kallikrein-kinin system, intrinsic pathway of coagulation, alternative complement pathway, renin-angiotensin system and fibrinolytic system. Recent in vivo studies have shown implications of PK for HAE, diabetic retinopathy, ICH and pathological thrombosis. Targeting PK may offer novel opportunities to treat these proinflammatory and prothrombotic disorders.

**Acknowledgements**

This work was supported in part by grants from Vetenskapsrådet (K2013-65X-21462-04-5), Hjärtsjukfonden (20110500), Stockholms läns landsting (ALF, 20120471), Cancerfonden (100615), and European Research Council grant (ERC-StG-2012-311575_F-12) to TR.

**Conflicts of interest**

None declared.

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