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The polyphosphate/factor XII pathway in cancer-associated thrombosis: novel perspectives for safe anticoagulation in patients with malignancies

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ABSTRACT

Cancer is an established risk factor for venous thromboembolism (VTE) and VTE is the second leading cause of death in patients with cancer. The incidence of cancer-related thrombosis is rising and is associated with worse outcomes. Despite our growing understanding on tumor-driven procoagulant mechanisms including cancer-released procoagulant proteases, expression of tissue factor on cancer cells and derived microvesicles, as well as alterations in the extracellular matrix of the cancer cell milieu, anticoagulation therapy in cancer patients has remained challenging. This review comments on a newly discovered cancer-associated procoagulant pathway. Experimental VTE models in mice and studies on patient cancer material revealed that prostate cancer cells and associated exosomes display the inorganic polymer polyphosphate on their plasma membrane. Polyphosphate activates blood coagulation factor XII and initiates thrombus formation via the intrinsic pathway of coagulation. Pharmacologic inhibition of factor XII activity protects mice from VTE and reduces thrombin coagulant activity in plasma of prostate cancer patients. Factor XII inhibitors provide thrombo-protection without impairing hemostatic mechanisms and thus, unlike currently used anticoagulants, do not increase bleeding risk. Interference with the polyphosphate/factor XII pathway may provide the novel opportunity for safe anticoagulation therapy in patients with malignancies.

Cancer and thrombosis

Cancer is an established risk factor for venous thromboembolism (VTE), which comprises both deep venous thrombosis (DVT) and pulmonary embolism (PE). Patients with all types of cancer experience increased incidence of thromboembolic events, commonly referred to as Trousseau's syndrome [1]. Indeed, more than 20% of all first VTE events are malignancy-associated and VTE is the second leading cause of death in patients with cancer [2]. The molecular basis for the association between malignant disease and thrombosis has been challenging to explore. Suggested mechanisms for cancer-associated procoagulant states include increased expression of the transmembrane protein tissue factor (TF) on cancer cell plasma membranes or circulating cancer-derived microvesicles, presence of a factor X–activating cysteine protease, tumor cell-derived mucinous glycoproteins that bind to P-selectin, and K-ras, EGFR, PML-RARA and MET oncogene activation [1]. In addition to driving cancer-associated thrombosis the coagulation system also contributes to tumor growth [3,4] and metastasis [5]. Anticoagulation therapy in cancer patients is challenging due to the high recurrence rate and increased rate of anticoagulant-related bleeding, compared to non-cancer patients [6]. Thus, new effective and safe anticoagulation strategies are needed for patients with cancer.

The Factor XII-driven contact system

Both arterial and venous thrombi are composed of activated platelets and varying amounts of fibrin. Fibrin formation can be initiated by TF (extrinsic coagulation pathway) or the plasma protein factor XII (FXII, intrinsic coagulation pathway). The intrinsic coagulation pathway is triggered by contact-induced auto-activation of zymogen FXII. Binding of FXII induces a conformational change that together with plasma kallikrein and high molecular weight kininogen leads to formation of the active serine protease (FXIIa). Activated FXII in turn drives fibrin formation via limited proteolysis of its substrate factor XI (FXI). Factor XII contact activation induced by the white clay material kaolin triggers plasma clotting in the diagnostic coagulation assay

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"activated partial thromboplastin time" (aPTT). Unlike other proteases of the coagulation cascade, deficiency in FXII is not associated with hemostatic abnormalities in humans and mice [7, 8]. In contrast, the FXII substrate FXI has a role in hemostasis in humans, however is dispensable for terminating bleeding in mice and other animals [9]. Challenging the concept of the coagulation balance, thrombus formation is defective in FXII deficient (*F12*$^{-/-}$) mice [10, 11]. Consistently, pharmacologic inhibition of FXIIa-driven coagulation provides thrombo-protection in extracorporeal bypass systems in large animals [12], in a collagen-coated arteriovenous-shunt in non-human primates [13] and in elective primary unilateral total knee arthroplasty in humans [14], without an increase in therapy-associated bleeding. Although FXII functions in thrombus formation in mice, large animals and nonhuman primates, there is a lack in epidemiologic studies that analyze a potential protection from thromboembolic disease in individuals with severe FXII deficiency. We have set up a registry including humans with severe FXII deficiency www.factor12.net and invite readers to register their "patients" with FXII deficiency in the database. In addition to coagulation, FXIIa initiates the kallikrein-kinin system that culminates in generation of the proinflammatory mediator bradykinin (BK) from its precursor high molecular weight kininogen. Binding of BK to the G-protein-coupled kinin B2 receptor (B2R) initiates intracellular signaling pathways that induce pain, increase vascular leakage, attract neutrophils and may directly facilitate tumor growth and angiogenesis [7, 15]. The principal mechanism underlying cancer-driven coagulation is thought to be elevated TF expression on cancer cells and secreted vesicles. However, experimental and clinical data indicate that additional mechanisms operate and contribute to cancer-associated thrombosis [16, 17]. Factor XII plasma levels are low in patients with lung [18], gastrointestinal [19] and colorectal [20] cancer, suggesting that FXIIa-driven pathways are active in patients with malignancy.

Polyphosphate

There has been renewed interest in the role of the contact activation pathway over the last decade following the seminal observation that FXIIa is critical for thrombosis. Misfolded protein aggregates, mast cell released heparin and high concentrations of extracellular RNA and DNA have been implicated as FXII contact activators *in vivo* [8]. Another important endogenous activator of FXII is the inorganic polymer polyphosphate (polyP). Polyphosphate is a linear macromolecule consisting of a few to several thousand residues of orthophosphate linked by phosphoanhydride bonds [21]. Short chain sodium polyP is soluble in biological fluids, however, the solubility of polyP decreases with an increase in chain length [22]. Polyphosphate is ubiquitously found in every prokaryotic and eukaryotic cell, where it has critical functions in energy metabolism [23]. The role of polyP in mammalian systems is not yet well described, however platelets are known to store the polymer in dense granules and release it upon activation [24]. Polyphosphate initiates coagulation in a FXII-dependent manner [25, 26] and amplifies various downstream coagulation mechanisms [21]. The activity of polyP for inducing FXII contact activation increases with chain length of the polymers [26]. FXII activation is linked to procoagulant platelets [27-30]. While *in vitro* data suggest that platelet-derived short chain polyP in disperse form has limited FXII activating capacity [26], the situation *in vivo* is more complex. In living systems, polyP precipitates in the presence of calcium ions and forms nanoparticles with decreased solubility [22]. Platelets store polyP together with high concentrations of calcium ions in their dense granules and platelet released polyP is bound to calcium [24]. Calcium binding markedly alters polyP procoagulant activities and potential for activating FXII. Short chain calcium polyP in nanoparticle form has greatly increased capacity for activating FXII compared to the disperse molecules in solution [22] indicating that aggregate formation rather than chain length determines procoagulant polyP activities. The polyP/FXII pathway operates independently of TF-mediated coagulation and triggers coagulation even in the absence of TF [31]. Infusion of phosphatase (an enzyme that degrades polyP) and polyP-binding agents efficiently interferes with blood clotting *in vitro* and thrombus formation in mouse models [32, 33]. Plasma clotting and thrombus formation is defective in mice and humans with genetic deficiency in platelet polyP [inositol hexakisphosphate kinase 1 null (*Ip6k1*$^{-/-}$) mice [34]; Hermansky-Pudlak Syndrome (HPS) [32]], respectively, similar to findings in *F12*$^{-/-}$ mice. Consistent with a selective function of polyP for thrombosis, targeting polyP interferes with occlusive clot formation while sparing hemostasis [33, 35]. The effect conferred by polyP targeting agents is reminiscent of the phenotype seen in *F12*$^{-/-}$ mice [10]. Infusion of polyP fails to initiate coagulation in *F12*$^{-/-}$ mice [32] supporting the notion that polyP operates via FXII *in vivo*. Taken together, polyP/FXII-initiated coagulation appears to be critical for thrombosis and interference with this pathway provides thromboprotection.

The polyP/FXII pathway in prostate cancer-associated thrombosis

Prostate cancer (PC) is the second most common cancer in men and the sixth leading cause of malignancy-associated mortality [36]. Although other malignancy types pose a higher relative risk for VTE [37], the high prevalence of PC makes PC-related VTEs a major medical burden. Prostate cancer cells secrete procoagulant TF-bearing microvesicles (prostasomes, specific exosomes) [38] that have a mean diameter of about 150 nm. Their cholesterol- and sphingomyelin-rich plasma membranes [39] are similar to exosomes secreted by breast, pancreatic, or colon adenocarcinoma cells [40, 41]. Elevated prostasome counts are often found in PC patient plasma [42] and are associated with elevated extrinsic coagulation pathway activation, indicating that plasma membrane TF is at least partly responsible for prostasome-triggered procoagulant activity [38]. While some clinical studies found increased TF activity in cancer patients with thrombosis [43-45], others failed to establish an association between TF activity and thrombosis in malignancy [46-48] indicating that in addition to TF other pathways exists in cancer patients. Recently, we investigated the procoagulant properties of PC cells and secreted prostasomes for FXII-dependent coagulation [49]. Prostate cancer cells and prostasomes expose polyP of chain length 200 to >1000mers on their plasma membrane. Prostate cancer-purified polyP potently activates FXII, triggers thrombin formation in a FXII-dependent manner and induces plasma clotting via the FXII-driven intrinsic pathway of coagulation. The polyP content correlates with procoagulant activity of the prostasomes. Both genetic and pharmacological targeting of either polyP or FXII confers resistance to PC-driven thrombosis in mice. Of note, inhibition of the polyP/FXII pathway in murine thrombosis models is not associated with any increase in therapy-associated bleeding. Inhibition of FXIIa with the humanized recombinant antibody 3F7 protects mice from PC-induced VTE and also reduces the increased procoagulant activity in PC patient plasma [12, 49].

In addition to polyP/FXII and consistent with increased TF-dependent procoagulant activity in plasma of PC patients [50], prostasomes and PC cells also initiate coagulation in a TF-dependent manner. While both FXII and TF initiate coagulation in cancer, inhibition of FXIIa was sufficient for blocking thrombosis, suggesting that TF activity is not sufficient for driving thrombosis in PC. Thrombus formation is a complex process that can be subdivided into initiating and propagating mechanisms. Following initiation of fibrin production, likely by TF and/or FXIIa on the plasma membrane, the FXIIa-dependent intrinsic pathway propagates fibrin formation within the developing