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Novel strategies of coagulation inhibition for reducing tumor growth and angiogenesis

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ABSTRACT

Activated proteins of the coagulation system are known to enhance angiogenesis and tumor growth. Notably, the main coagulation proteins involved are those of the extrinsic and common pathways. Strategies to attenuate tumor progression by decreasing activation of the coagulation system may be compromised by an increased risk of bleeding. Currently, studies using derivatives of heparins devoid of or with low anticoagulant activity are being conducted. Heparanase protein was demonstrated to enhance tissue factor activity. In-house developed peptides, deriving from tissue factor pathway inhibitor 2 (TFPI-2) were shown to inhibit heparanase procoagulant activity by interrupting the interaction between TF and heparanase. These peptides appeared to have a non-hemostatic anti-angiogenic effect. In a mouse model, the peptides caused a significant reduction in tumor growth and relapse, without predisposing to bleeding. Hence, the effects of these inhibitory peptides in cancer patients may deserve further investigation in clinical research studies.

1. Introduction

From the phylogenetic standpoint, cancer and the coagulation system evolved simultaneously about 450 million years ago [1], implying a potential interaction between these major biological pathways. Rickles et al. recently reviewed published data regarding an association between tissue factor (TF), thrombin, fibrin and cancer development [2]. These coagulation proteins were shown to stimulate tumor growth either through direct enhancement of the coagulation system or through indirect, non-hemostatic effects (Fig. 1). Notably, there is paucity of information about involvement of the intrinsic coagulation system in tumor growth. From the anti-tumor therapeutic perspective, inhibition of one or more of the coagulation proteins, while affecting tumor development, may trigger bleeding complications. There is much uncertainty regarding the likelihood that the protein domain involved in activation of the coagulation system is identical to that involved in boosting tumor growth.

2. Inhibition of tissue factor and factor VIIa

Immunohistochemical studies have revealed that many tumors express high levels of TF, raising the possibility of TF role in the pathogenesis of cancer [3,4]. This protein was demonstrated to be involved in tumor growth *via* activation of the coagulation system as well as *via* non-hemostatic effects [5–7]. Suppression of tumor metastasis by impairing FVIIa binding to its cellular receptor TF was previously shown to induce various intracellular signaling events, which were thought to be responsible for TF-mediated biologic effects, including angiogenesis and tumor metastasis reduction [6]. Clinical trials using anti-TF or anti- TF/VIIa drugs are sparse. A recent phase I study determining pharmacokinetic and safety profiles of an anti-TF antibody ALT-836 in subjects with acute lung injury or acute respiratory distress syndrome reported no serious bleeding events [8]. Of note, in several other studies, recombinant nematode anticoagulant protein c2 (NAPc2), a potent inhibitor of the TF/factor VIIa complex, was demonstrated to have anti-metastatic effects [9,10], potentially paving the way for evaluation of this product as an anti-cancer drug.

3. Heparins as anti-tumor drugs

In the coagulation system, heparins mainly inhibit thrombin and factor Xa activity. The underlying mechanisms of heparin-associated anti-tumor effect include attenuation of the coagulation system, heparanase inhibition, release of growth factors from the endothelial cell surface and blocking of P- and L-selectin mediated cell adhesion. The interaction of heparins with proteins, such as growth factors and selectins, is non-specific, imposing a shortcoming on these molecules, since anti-tumor proteins, such as tissue factor pathway inhibitor (TFPI), could also be potentially released. The lack of target selectivity could reduce the benefit of using heparins as anti-tumor drugs.

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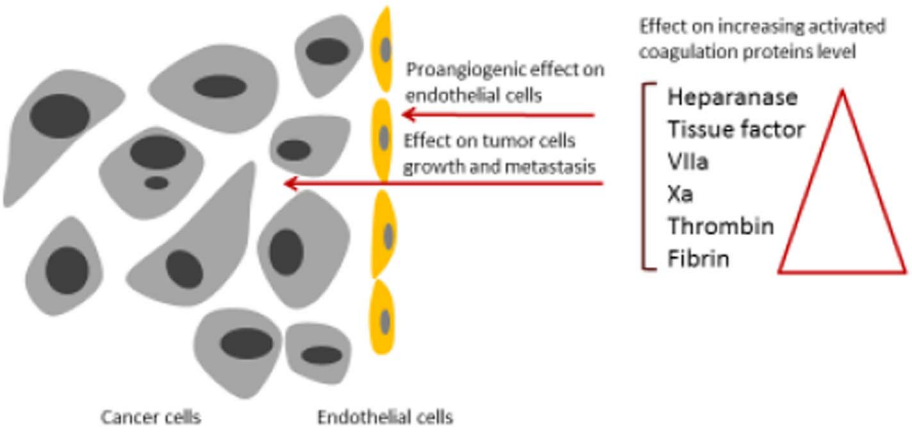

Fig. 1. Coagulation proteins are involved in enhancement of tumor growth through a direct effect on endothelial and tumor cells. An indirect effect of increasing levels of downstream activated coagulation proteins may further enhance tumor growth.

Heparins derived from an animal source, including the unfractionated heparin (UFH) and biochemically derived low molecular heparins (LMWHs), exhibit an anticoagulant effect, which is an advantage in terms of attenuation in the activation of other coagulation factors (*e.g.*, thrombin and fibrin), while being a disadvantage in terms of their potential to induce bleeding.

A recently published Cochrane analysis assessed the efficacy and safety of heparins in ambulatory cancer patients. The 15 randomized controlled trials (RCTs) included in the analysis, enrolled 7622 participants with available follow-up data. In each of these RCTs, either UFH or LMWH was used, demonstrating a minor effect on 12-month and 24-month mortality, with a decrease in venous thromboembolism (VTE) along with a likely increase in minor bleeding. Potential survival improvement associated with different types of anticoagulants should be further evaluated in correlation to a specific type and stage of cancer. Currently, decision-making regarding the use of heparin in a particular cancer patient should be based on the assessment of an individual risk-benefit balance with consideration of patient's preferences [11].

Heparin mimetics are synthetic and semi-synthetic compounds that are highly sulfated, structural analogues of glycosaminoglycans. The heparin mimetic PI-88 (Progen Pharmaceuticals Ltd. Brisbane, Australia) is the product of exhaustive sulfation of the oligosaccharide phosphate fraction of the extracellular phosphomannan derived from the yeast Pichia (Hansenula) holstii NRRL Y-2448 [12]. PI-88 was studied in a phase I/II study, including a total of 172 patients with hepatocellular carcinoma (HCC) who were randomized to three groups: one untreated arm (Group A) and two PI-88 arms (Group B: 160 mg/day; Group C: 250 mg/day). PI-88 was administered over nine 4-week cycles, followed by a 12-week treatment-free period. Treatment-related adverse effects included thrombocytopenia, injection site hemorrhage, partial thromboplastin time (PTT) prolongation and hepatotoxicity. Four serious adverse events were possibly related to PI-88 treatment: one (1.8%) was in group B patients and six (10.5%) were in group C [13]. In the follow-up study, a total of 143 patients (83.1% of the 172 participants in the first phase II study) participated. Patients in the treatment group demonstrated a delay in the onset and a decrease in the frequency of HCC recurrence that provided a clinically significant survival advantage for up to 3 years after treatment, compared with those of the control group [14]. This product is being currently evaluated in a phase III clinical study in patients with HCC.

In an open-label, multicenter, phase II study of PI-88 in advanced melanoma, patients received a subcutaneously injection for four consecutive days followed by three drug-free days per week in a 28-day cycle. A total of 44 patients were enrolled in the intent-to-treat population, with 59.1% having received previous chemotherapy. Serious bleeding occurred in two patients and three patients developed a positive anti-platelet antibody test during the study. Results demonstrated an overall survival and time-to- progression similar to standard chemotherapy. The authors concluded that while the study failed to meet the primary end-point of progression-free-survival of $\geq 20\%$, some evidence of PI-88 activity was observed, requiring further investigation [15]. Following these results, Progen Pharmaceuticals Ltd. designed a series of second-generation versions of PI-88 compounds named PG500. The lead molecule of this series PG545, exhibiting low anticoagulant properties and potent inhibition of heparanase, is being currently investigated in a phase I clinical trial [16].

Glycol split heparins are synthetic short length heparins that are devoid of or have low anticoagulant effect but maintain other heparin properties of inhibiting heparanase and releasing growth factors from the endothelial cell surface. SST0001 (Roneparstat, Sigma-Tau Pharmaceuticals, Gaithersburg, MD, USA) is a low anticoagulant 100% *N*-acetylated glycol-split heparin, obtained from standard porcine mucosal heparin and acting as a potent heparanase inhibitor. This compound is currently in phase I study in advanced multiple myeloma [17].

Momenta Pharmaceuticals has presented anti-metastatic preclinical data on a heparin mimetic, M402 (Necuparanib), that is a low molecular weight heparin, resulting from depolymerization of heparin and further oxidation and borohydride reduction. *In vitro* and *in vivo* studies of M402 compound showed reduced anticoagulant activity and inhibition of tumor metastasis [18]. Currently, M402 is in phase II clinical trial in patients with pancreatic cancer.

4. Effects of oral anticoagulants on tumor progression

A recent Cochrane analysis including seven randomized control trials, evaluated the effect of anticoagulation (using warfarin in six and apixaban in one trial) on survival of cancer patients. The use of oral anticoagulants in cancer patients was not found to be associated with an improved mortality rate, while an increase in the bleeding risk was revealed [19].

5. Inhibition of heparanase procoagulant activity

Heparanase is an enzyme capable of cleaving heparan sulfate (HS) side chains in a limited number of sites, yielding HS fragments of still appreciable size (~5–7 kDa) [20,21]. Heparanase activity was reported to correlate with the metastatic potential of tumor cells, attributed to enhanced cell dissemination as a consequence of HS cleavage and remodeling of the extracellular matrix (ECM) barrier [22,23]. Similarly, heparanase activity was implicated in neovascularization, inflammation and autoimmunity, involving migration of vascular endothelial cells and activated cells of the immune system [22–24]. Up-regulated expression of heparanase was noted in essentially all human tumors examined, as well as in inflammation, wound healing and diabetic nephropathy [22–24]. A single human heparanase cDNA sequence was independently reported by several research groups [25–28]. Thus,