Tissue factor pathway inhibitor and cancer

[haematologica reports]
2005;1(9):12-14

GRUEL Y
ROLLIN J
REGINA S
IOCHMANN S
HUBE F
REVERDIAU P

Department of Hematology, Hemostasis and Inserm U 618, Tours, France

The coagulation protease cascade and the fibrinolytic system are leading to the synthesis of two serine-proteases that are essential for normal hemostasis but these enzymes also play important regulatory roles in angiogenesis, cancer cell migration and tumor growth.

Tissue factor (TF) is the physiological trigger of coagulation cascade and is mainly regulated by TFPI-1 (tissue factor pathway inhibitor), a kunitz-domain serine protease inhibitor. But, another protein analogous to TFPI-1 and named TFPI-2 is also present in large amounts in extracellular matrices (ECM), and has been identified as playing an important role in cancer invasion.

TF a 47kD-glycoprotein structurally related to cytokine receptors, and is expressed by many normal cells present in vessel wall.[1] TF is the physiological trigger of the coagulation cascade by serving as the cofactor for VII/VIIa.[2] TF-VIIa complex thus activates coagulation factors X and IX and initiates hemostasis through downstream thrombin generation, resulting in fibrin formation and platelet activation. TF can also synthesized by several types of cancerous cells and this expression is likely contributing to the progression of some tumors on one hand,[3] and to the prothrombotic states associated with many cancers on the other hand.

TF expression by tumor cells enhances the efficiency of experimental metastasis.[3,4] Among the processes that are involved, interactions of the TF cytoplasmic domain with the mobility-enhancing actin-binding protein 280, and the formation of proteolytically active TF/VIIa complexes on the tumor cell surface, were shown to be potentially important for cancer cell adhesion and migration.

TF is also contributing in vascular development and is induced in angiogenic endothelial cells. Up-regulation of TF in angiogenesis leads to a more procoagulant vascular surface, but this shift is balanced by anticoagulant mechanisms, involving TFPI-1, expressed by endothelial cells, and which likely plays an important in maintaining a patent vascular bed within tumors.

TFPI-1 physiologically regulates the protease activity of TF-VIIa complex on the endothelium,[5] and is typically associated with glycosyl-phosphatidyl inositol-anchored receptors on the cell surface. TFPI-1 consists of three Kunitz-type inhibitory domains and a basic, proteoglycan-binding COOH terminus. TFPI-1 locks TF into an inactive TF-VIIa-Xa-TFPI-1 complex by binding simultaneously to factors VIIa and Xa. Apart from this major role that inhibits thrombin generation, TFPI-1 is also likely serving as an adhesive ligand for cancer cells to extracellular matrices (ECM). It was indeed demonstrated that cancer cells directly adhere to TFPI-1 present in ECM,[6] and this process enhanced by heparin, solely depends on TF/VIIa/TFPI-K1 domain interactions, while it does not implicate factor Xa. Importantly, this TF/VIIa/TFPI-1 interaction also promotes cell migration. Apart from its role in cancer cell adhesion, TFPI-1 was also recently shown to inhibit the endothelial tube formation induced by either FGF-2 or TF/VIIa, in a concentration-dependent manner.[7]

Cancer is undoubtedly a clinical situation well identified as being associated with a high risk of recurrent thrombosis, particularly when disseminated with metastases. One of the mechanisms that are considered to explain the prothrombotic state of patients with cancer is the increase of TF expression by the cancerous cells themselves.[8] A relative decrease of plasma TFPI-1 levels could therefore be a risk factor for venous thromboembolic events in cancer patients. But until now, this hypothesis has not been specifically investigated in this category of patients. In contrast, It has been shown that cancer patients with solid tumors have increased plasma levels of both free and total of TFPI-1, whereas those with leukemia and related blood malignancies have normal levels of this inhibitor. High plasma levels of FXa/TFPI complexes have also been measured in these patients.[9]

However, according to a large population-based case-control study, low TFPI-free and total antigen levels constitute a risk factor for deep vein thrombosis.[10] It would be therefore of interest to focus on cancer patients to evaluate the potential influence of TFPI-1 levels and plasma distribution on the risk of thrombosis in this particular clinical context.

TF can also be inhibited on the cell surface by TFPI-2 *in vitro*, but the extent of this effect is dramatically lower than those achieved by TFPI-1. TFPI-2 has been identified originally by homology to TFPI-1, as a placental trypsin inhibitor (and was thus previously named PP5 for placental protein 5), or as a matrix-associated serine protease inhibitor (MSPI).[11] TFPI-2 binds to the extra cellular matrix through ionic interactions involving the basic COOH terminus and the K3 domain of TFPI-2. Potential binding sites in the matrix are proteoglycans, as well as certain ECM proteins, such as collagen I, vitronectin, and laminin 5.

TFPI-2 is a potent inhibitor of plasmin *in vitro*, whereas it exhibits a low inhibitory activity towards TF-VIIa complex.[12] Therefore, there is no evidence that TFPI-2 regulates the TF pathway at physiological levels. In this regard, it has been showed that TFPI-2 does not affect TF-dependent procoagulant activity of aggressive melanoma cells.[13]

By inhibiting plasmin, TFPI-2 has been demonstrated to effectively decrease the activation of several metalloproteases such as MMP-1, MMP-3 and MMP-9,[14] and to reduce the invasive potential of several tumor cell lines. During tumor progression, malignant cells may invade adjacent tissues, particularly lymph nodes, and increased plasmin activity in the vicinity of malignant cells, with subsequent activation of MMPs and ECM degradation, enhances tumor invasion and metastases.[14-16]

TFPI-2 gene is now considered as being a tumour suppressor gene. In this regard, transcriptional silencing of the TFPI-2 gene by promoter hypermethylation has recently been demonstrated in in several cancer cell lines derived from choriocarcinoma,[17] glioma,[18] fibrosarcoma, breast and prostate cancers.[19] A relative decrease in TFPI-2 mRNA synthesis within tumors was then demonstrated in several cancers i.e. in human gliomas,[16] non-small cell lung cancers (NSCLC)[20] and in the majority of pancreatic cancers.[21] Moreover, TFPI-2 protein is undetectable by western blotting in high-grade glioblastomas. TFPI-2 protein synthesis studied by immunohistochemistry in different other tumours (laryngeal, breast, gastric, colon, pancreatic, renal and endometrial cancer) was also shown to decrease when the degree of malignancy increased.[22] In NSCLC, hypermethylation of the TFPI-2 gene promoter was also demonstrated by restriction enzyme-PCR in 30% of NSCLC, partly explaining that tumour TFPI-2 gene expression was decreased.[20] Moreover, this hypermethylation was more frequently found in patients lymph node metastases, The role of TFPI-2 in cancer invasion was recently supported by injecting fibrosarcoma cell lines expressing active or inactive forms of TFPI-2 in mice.[23] In addition to its role in regulating MMP activation, human TFPI-2 could also regulate tumour angiogenesis by reducing synthesis of the VEGF receptor and affect the expression of several genes involved in oncogenesis, invasion and apoptosis.

Both TFPI-1 and TFPI-2 do exhibit high affinity to glycosaminoglycans and thus to unfractionated heparin (UFH) or low-molecular weight heparins (LMWH). This explains why injections of UFH or LMWH induce the release of TFPI-1.[24] On the other hand, heparins are likely interfering with various processes involved in tumor growth, angiogenesis and metastasis and their use could be associated with a prolonged survival in cancerous patients. Therefore, the role of TFPI-1 and TFPI-2 regarding these biological and clinical effects of GAG deserves to be further studied.

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