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Review

Cancer therapy targeting the fibrinolytic system☆

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ABSTRACT

The tumor microenvironment is recognized as a key factor in the multiple stages of cancer progression, mediating local resistance, immune-escape and metastasis. Cancer growth and progression require remodeling of the tumor stromal microenvironment, such as the development of tumor-associated blood vessels, recruitment of bone marrow-derived cells and cytokine processing. Extracellular matrix breakdown achieved by proteases like the fibrinolytic factor plasmin and matrix metalloproteases is necessary for cell migration crucial for cancer invasion and metastasis. Key components of the fibrinolytic system are expressed in cells of the tumor microenvironment. Plasmin can control growth factor bioavailability, or the regulation of other proteases leading to angiogenesis, and inflammation. In this review, we will focus on the role of the fibrinolytic system in the tumor microenvironment summarizing our current understanding of the role of the fibrinolytic factors for the modulation of the local chemokine/cytokine milieu, resulting in myeloid cell recruitment, which can promote neoangiogenesis.

Contents

Abbreviations: CXCL5, chemokine, CXC, motif, ligand 5; EACA, lysine derivatives epsilon-aminocaproic acid; ECM, extracellular matrix; FDP, fibrin degradation product; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; IBD, inflammatory bowel disease; IL, interleukin; ILK, integrin linked kinase; LRP, low-density lipoprotein (LDL) receptor-related protein; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PA, plasminogen activator; PAI-1, plasminogen activator inhibitor-1; PLG, plasminogen; TA, tranexamic acid; TLR, Toll like receptor; TNF-α, tumor necrosis factor-α; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; VEGF, vascular endothelial growth factor; YO-2, Trans-4-Aminomethylcyclohexanecarbonyl-Tyr (O-Pic)-octylamide.

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1. Introduction

Cancer research has long focused solely on the cancer cell itself, and the mutations in genes leading to altered cell behavior including uncontrolled cell growth. Although the identification of important genes supporting or suppressing tumor growth increased our understanding of the various cancer cell types, recent studies demonstrated that cancer cells establish a microenvironment to support their own growth and enhance metastasis. Microenvironmental cells include endothelial, stromal cells and leukocytes just to mention a few of them. These microenvironmental cells, can enhance tumor progression and drug-resistance, but also help cancers to evade the regulation of the immune system. The microenvironment also includes extracellular matrix proteins, protease, chemokines and cytokines. Studies highlighting the significance and functions of cells of the microenvironment are slowly emerging.

Proteases belong to several families of enzymes that catalyze protein breakdown. Around 2–4% of a typical genome encodes proteolytic enzymes [1]. Protease can be classified into serine, threonine, cysteine, aspartate, glutamic acid proteases, and matrix metalloproteinases (MMPs). The activation of fibrinolytic factors or MMPs seems to be associated with the growth and metastasis of certain tumors, and can even be enhanced after the administration of anticancer drugs [2] within cells of the tumor microenvironment, also known as the tumor niche.

1.1. Regulation of serine protease plasmin generation and its inhibition

The central molecule of the fibrinolytic system is one of the serine proteases plasmin (Fig. 1). Plasminogen (PLG), an inactive form of plasmin is produced in the kidney and liver [3]. PLG can be rendered into its active form plasmin through the tissue-type plasminogen activator (tPA) or urokinase plasminogen activator (uPA).

tPA is believed to activate PLG during fibrinolysis, whereas uPA activates cell-associated PLG. Plasmin activity is directly inhibited by α2-antiplasmin and α2-macroglobulin or can be indirectly controlled on the level of plasminogen activators (PAs) by PA inhibitor (PAI) type 1 and type 2. PLG binds to fibrin, to generate soluble fibrin degradation products [4], causing fibrinolysis (Fig. 1).

Coagulation and fibrinolysis are well coordinated under physiological conditions and insure blood flow, while preventing blood loss, and guarantying the timely removal of ongoing or acutely induced fibrin deposits. Plasmin can both activate and inactivate coagulation factors V and IX. Plasmin activation provides a broad spectrum of reactions including proteolytic activity, cell migration and signaling pathway activation, which are involved in both physiologic and pathologic processes such as inflammation, thrombosis and cancer. These feedback mechanisms ensure the balance between the fibrinolytic and the coagulation system. Plasmin can be activated either on a fibrin-containing thrombus or on cells.

In addition, PLG can be trapped at the cell surface by PLG receptors, like low-density lipoprotein receptor-related protein-1 (LRP-1) or annexin A2 (Fig. 2). Annexin A2 is expressed on various cell types, including endothelial cells, monocytes, macrophages and cancer cells. Plasmin/PLG binds to monocytes via its receptors annexin A2 and PLG-R_{KT} [5]. Studies with the annexin A2-deficient mouse have suggested important functions for annexin A2 and the heterotetramer in fibrinolysis, in the regulation of the LDL receptor and in cellular redox regulation (Fig. 2). Annexin A2 is a pleiotrophic calcium- and anionic phospholipid-binding protein that exists as a monomer and as a heterotetrameric complex with the PLG receptor protein, S100A10, also known as p11 [6]. P11 binding to PLG mediates the activation of uPA or tPA as this binding facilitates the conversion of PLG to plasmin [7].

Aside from the activation of uPA due to its binding to fibrin, uPA activation can also be achieved after the binding of the pro-uPA molecule to the uPA receptor (uPAR; CD87) on the cell surface under physiological conditions [8] (Figs. 1, 2). The binding of single chain uPA to uPAR strongly enhances PLG cleavage to generate active plasmin. A positive feedback is produced since plasmin, by a proteolytic cleavage of the Lys158-Ile159 peptide bond, converts latent single chain-uPA to an active two-chain uPA [9].

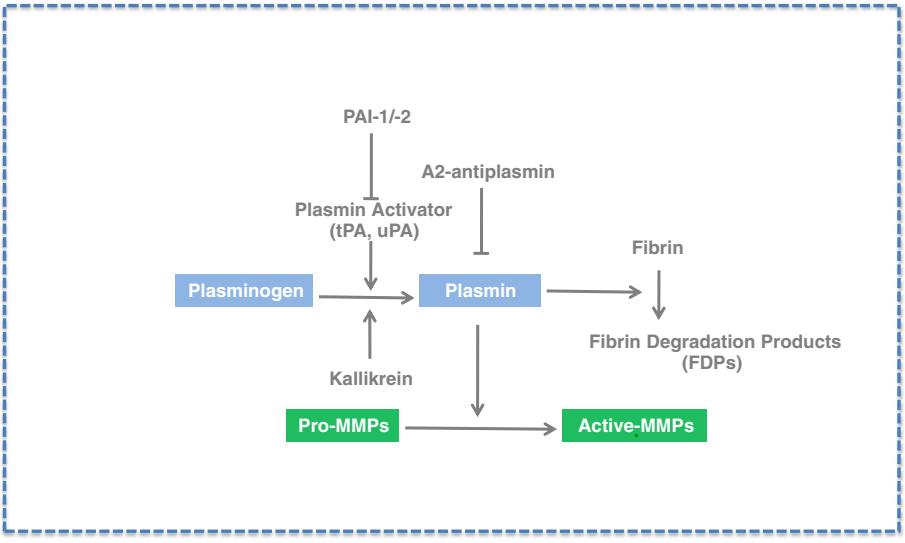

Fig. 1. The proteolytic environment induced by fibrinolytic factors. PLG can be converted to its active form plasmin by both tPA and by uPA or kallikrein. Plasmin can degrade fibrin into fibrin degradation products. Plasmin activates pro-MMPs into active MMPs and can activate pro-uPA into uPA. Plasmin activation is regulated by inhibitors of PLG activation, such as PAI-1, -2, and by inhibitors of plasmin itself, such as a2-antiplasmin or a2-macroglobulin.

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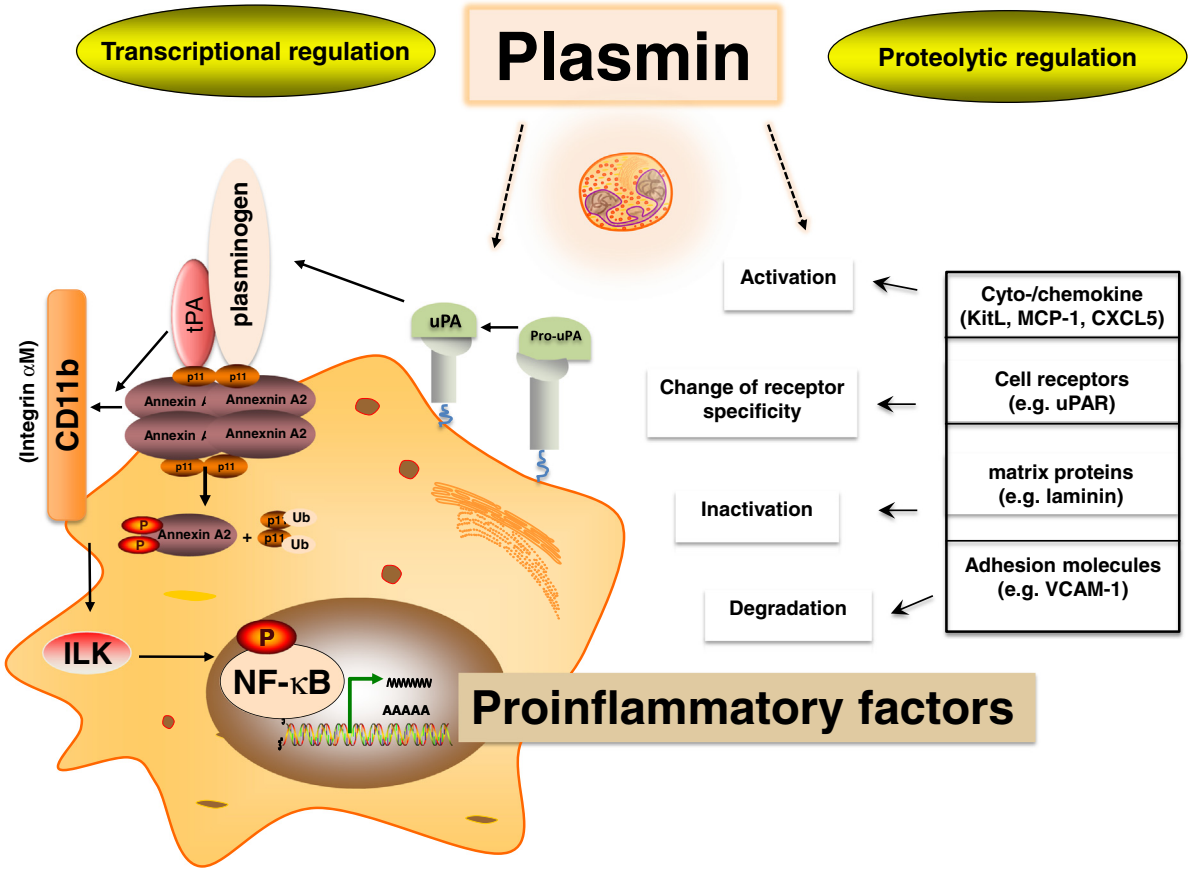

Fig. 2. Plasmin creates a proinflammatory microenvironment. Myeloid cells are part of the stromal compartment within the microenvironment in tissues undergoing remodeling as found during cancer growth. 1) Plasmin can modulate inflammatory cell influx and expansion on the transcriptional level via NF-κB activation: Myeloid cells of the microenvironment like monocytes and neutrophils bind PLG as well as tPA and/or uPA either via Annexin/p11 or e.g. by uPAR. Plasmin cleaves both tPA and uPA, transforming them from single chain to more active two-chain polypeptides through a positive feedback mechanism. tPA after binding to the Annexin A2 complex can interact with the integrin αM leading to ILK translocation and NF-κB activation. This leads to the expression of NF-κB proinflammatory factors. 2) Pericellular proteolysis mediated by uPAR and its ligand uPA helps to disrupt the ECM by altering the proteolytic balance within the microenvironment favoring either the activation of e.g. MMPs or the secretion of tissue inhibitor of MMPs via plasmin generation, or breaking of cell–matrix interactions with receptors, such as integrins. Activation of fibrinolytic factors changes the bioavailability and activation status of growth factors or release bioactive fragments of ECM molecules and growth factors that regulate migratory behavior of inflammatory cells.

Aside from endogenous inhibitors of the fibrinolytic pathway like PAI-1 or α2-antithrombin, fibrinolytic inhibitors have entered the clinic several decades ago. Today only synthetic products are on the market, namely lysine derivatives epsilon-aminocaproic acid (EACA), and the more potent tranexamic acid (TA) [10]. Clinical applications for the administration of fibrinolytic inhibitors include bleeding prevention. Treatment with synthetic fibrinolytic inhibitors is effective and safe as demonstrated in a large number of controlled clinical trials.

The fibrinolytic inhibitors EACA and TA reduce PLG's binding to fibrin by binding reversibly and in a competitive manner to the lysine binding site of PLG. This reduces the activation of PLG to plasmin. Thus, EACA and TA are indirect plasmin inhibitors.

It was shown that aprotinin, a direct inhibitor of plasmin and other proteases like kallikrein, which had been used in cardiac surgery and liver transplantation, was removed from the market in 2008 because severe side effects (increased mortality) was reported in the BART study [11].

Trans-4-Aminomethylcyclohexanecarbonyl-Tyr (O-Pic)-octylamide (YO-2) is a newly developed plasmin inhibitor. YO-2 can block the active center of plasmin and causes apoptotic cell death of M1 (melanoma) and HT29 colon carcinoma cells [12]. The tumor growth inhibitory effect of YO-2 was studied using HT-29 human colon carcinoma, HT-18 human melanoma and HT-58 human B cell lymphoma inoculated as xenografts into immuno-deprived mice [13]. We were able to show that the plasmin inhibitor YO-2 blocked inflammation and lymphoma growth by blocking myeloid cell influx in vivo [14–16].

1.2. Fibrinolytic factors expressed in cells of the tumor microenvironment can control MMP activity

During cancer progression, dynamic remodeling of the extracellular matrix (ECM) is necessary for cell migration. ECM remodeling is primarily accomplished by controlling the expression or activities of ECM enzymes like MMPs, and the PA system [17,18]. When human tissue samples of breast, skin and colon cancer were immunohistochemically analyzed, uPA, tPA, PAI-1 and uPAR were mainly expressed in the cells of the tumor microenvironment including fibroblasts, endothelial and hematopoietic cells, but not in tumor cells [19]. High uPA expression in tumor tissues as compared with normal tissues has long been noted [20,21]. Increased uPA levels found in breast cancer patients correlate with poor relapse-free and overall survival [22] and are indicative of metastasis in various cancers [23].

Plasmin can degrade basement membrane proteins, such as thrombospondin, laminin, fibronectin and fibrinogen in vitro. In addition, plasmin can degrade additional components of the ECM through its ability to convert some pro-MMPs to active MMPs, including MMP-1, MMP-2, MMP-3 and MMP-9 [24–26]. These matrix

proteins include collagens, laminin, fibronectin, vitronectin, elastin, aggrecan and tenascin C [4].

Plasmin is involved in other aspects of MMP signaling besides their activation. Thus, once activated, MMP-2, -3, -7, -9, and -12 can initiate a negative feedback signal, by, for example, degrading PLG [27,28]. Degradation of plasmin-suppressing serpin proteinase inhibitors (e.g. α2-antiplasmin [29]) by MMPs (e.g. MMP-3), which promotes the conversion of pro-MMPs, is an example of how the fibrinolytic and MMP pathway control each other.

2. Plasmin expands myeloid cells

Leukocytosis, granulocytosis, thrombocytosis, and erythrocytosis are clinical features associated with the so-called "paraneoplastic syndromes," which include signs and symptoms affecting sites distant from the tumor or its metastases. Such syndromes are not caused by invasion, obstruction, or bulk mass of the tumor but are instead due to inappropriate secretion of cytokines, growth factors, and hormones [30]. Leukocytes like myeloid, other inflammatory cells, stromal cells (e.g. tumor-associated fibroblasts), or endothelial cells are just some of the cells found in the tumor microenvironment. Myeloid cells comprising macrophages, neutrophils and dendritic cells are generated from hematopoietic stem cells and are important cells of the innate and adaptive immune system [31].

Macrophages are potent producers of many proteases, including cathepsins, MMPs, and serine proteases [32]. uPA deficient mice showed impaired metastasis in a transgenic mammary cancer [33]. In this model uPA was mostly produced by macrophages and in a transgenic breast cancer model uPA loss inhibits metastasis. Activation of these proteases is associated with invasive and chemo-resistant cancer phenotypes (for review see Dano et al. [19]).

Granulocyte-colony stimulating factor (G-CSF) induces stem cell mobilization and an increase in circulating myeloid cells. Two groups could show that plasminogen is indispensible for this process [34,35].

Kit ligand (KitL, also known as stem cell factor) can expand myeloid cells and hematopoietic progenitor cells [36]. We have shown that the successive activation of plasminogen and MMP-9 ultimately leads to the release of the hematopoietic growth factor KitL and myeloid cell expansion [26].

2.1. Plasmin regulates proinflammatory cytokines and inflammation by activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway

Several groups have demonstrated that plasmin contributes to inflammation by stimulating the gene expression/production of cytokines, reactive oxygen species, and other mediators [37]. Activation of the NF-κB pathway promotes proinflammatory cytokine and chemokine production in disease tissues [38]. Much attention has focused on the development of anti-inflammatory drugs targeting NF-κB [39].

NF-κB activation can occur by two pathways: The canonical NF-κB pathway has been defined primarily in response to tumor necrosis factor-α (TNFα) and interleukin-1α (IL-1α) signaling, typical proinflammatory cytokines that drive chronic inflammation. Activation of this pathway leads to activation of RelA that regulates expression of proinflammatory and cell survival genes. The alternative NF-κB pathway is activated by lymphotoxin-β, CD40L, B-cell activating factor, and receptor activator of NF-κB ligand, but not TNF-α, and activates the RelB/p52 complexes.

tPA-mediated NF-κB activation increases the expression of proinflammatory chemokines in macrophages (for review see Lin et al. [40]). The fibrinolytic factor tPA has been shown to activate NF-κB after binding to the membrane receptors LRP-1 or annexin A2. Under physiological conditions, NF-κBs are retained in the cytoplasm by its specific inhibitor, IκB [41]. When tPA binds to Annexin A2 in macrophages, NF-κB is translocated to the nucleus (Fig. 2). Receptor binding of tPA leads to phosphorylation of IκB that causes its own degradation and the release of NF-κB dimers p65/p50 (canonical activation) or RelB/p52 (non-canonical activation) into the nucleus resulting in the subsequent DNA binding and transcription of target NF-κB activation [41].

Another way for tPA do induce NF-κB is by promoting the aggregation of annexin A2 and integrin CD11b (also known as integrin αM) expressed e.g. on myeloid cells and upregulated during inflammation leading to the clustering and activation of integrin CD11b signaling, which in turn activates its downstream integrin linked kinase (ILK) and phosphorylates IκB. In addition, plasmin supplemented monocyte cultures induce concentration-dependent mRNA expression of TNF-α, IL-1α, IL-1β, monocyte chemoattractant protein-1 (MCP-1), and tissue factor [42–45].

tPA can also activate NF-κB after binding to LRP-1 as shown in models of cerebral ischemia. Zhang et al. found that tPA-mediated NF-κB activation was blocked after LRP inhibition [46].

Finally, He et al. showed that tPA binding to Annexin A2 enhances the generation of active plasmin. Plasmin then can generate a monomer of Annexin A2 separates Annexin A2 from p11 and prevents further plasmin generation. This modified monomer of Annexin A2 associates with Toll like receptor-4 (TLR-4) and leads to the activation of protein kinase C activation on endothelial cells. We recently showed that *IL-1β*, *TNF-IL-1α*, *IL-6* and *MCP-1* (also known as CCL2) in monocytes in a dose-dependent manner [15].

2.2. Plasmin regulates inflammation by changing the chemokine-/cytokine signaling pathways

Clinical and experimental data denote a link between chronic inflammation and tumor. Malignancies can develop after chronic inflammation through the exposure of proinflammatory cytokines and sustained activation of signaling pathways such as NF-κB and STAT3. Cytokines and chemokines like IL-1, IL-6, TNF-α, and MCP-1 are often associated with tumor progression and can be produced either by tumor or microenvironmental cells [47]. Plasmin has recently been depicted as being a proinflammatory cell activator because it can modify inflammatory cell migration and inflammation [37,48–50]. In accord with this concept, we showed that the fibrinolytic system is activated in the early phase of acute graft versus host disease, an immunological disease associated with a cytokine storm that can occur after allogeneic cell transplantation [15]. Plasmin inhibition rescued mice from the lethal consequences of acute graft versus host disease and the mounting of the disease-associated cytokine storm.

But how can plasmin sustain a proinflammatory condition? As discussed before, plasmin can regulate the gene expression of proinflammatory cytokines/chemokines through NF-κB activation. In addition, plasmin by virtue of its proteolytic activity can cause cleavage/shedding of biomolecules. Secreted proteases, like MMPs and plasmin regulate the repertoire of available extracellular growth factors by enzymatic activation, inactivation, or degradation [51], and enhance ECM remodeling necessary for cell migration (Fig. 2). ECM proteins also can establish a stable gradient of growth factors [16,52,53]. Proteases like MMPs and plasmin can shed or cleave adhesion and growth factor receptors from the surface of cells [54] (Fig. 2). Plasmin can convert the latent form of growth factors into its active form as for transforming growth factor-beta (TGF-β) [55], or platelet-derived growth factor C [56], or adhesion markers like vascular cell adhesion protein-1 (VCAM-1). Accelerated shedding of molecules like syndecan-1 and -4, can anchor cells to the ECM components [57]. This shedding can either be direct or via the activation of MMPs.

Another protein that is regulated by plasmin is MCP-1. MCP-1 can recruit myeloid cells like monocyte/macrophage, memory T cells and dendritic cells in murine inflammation models [58,59]. Plasmin can enhance MCP-1 signaling (a) by enhancing NF-κB mediated MCP-1 transcription (see Section 2.1), and (b) by releasing a MCP-1 fragment with improved chemoattractive ability due to its proteolytic capacity

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(post-translational modification) [15,60,61]. We showed that plasmin activation enhanced the MCP-1 chemoattractive ability of $CCR2^+$ inflammatory cells [15].

Tumor and/or stromal cells synthesize MCP-1. CCR2 is the receptor of MCP-1. MCP-1 and PLG recruit inflammatory $CCR2^+$ macrophages to tumor sites [62], with subsequent infiltration as metastasis-associated macrophages in the metastasizing tumors. Lack of MCP-1 signaling was shown to impair macrophage infiltration in a model of cervical carcinogenesis [63]. Tumor-associated macrophage infiltration in mammary tumors was induced by MCP-1 and correlated with the stromal MCP-1 expression levels. MCP-1-driven macrophage infiltration contributed to tumor progression [64,65]. It is therefore possible that plasmin by modulating critical tumor-associated chemokine changes the myeloid landscape of the tumor microenvironment.

Inflammatory bowel disease (IBD) can be divided into two major disorders, Crohn's disease and ulcerative colitis. Patients with IBD are at increased risk for colorectal cancer. The cancer risk is related to the duration and anatomical location of plasmin activation occurring in the early phase of IBD in mice [16]. The overall prevalence of colorectal carcinoma in any ulcerative colitis patient, based on 116 studies, was estimated to be 3.7% [66].

The influx of $CD11b^+$ macrophages and neutrophils into inflamed tissues is critically involved in the pathogenesis of colitis. Genetic and pharmacological inhibition of plasmin prevented IBD progression in various murine models of IBD [16]. Chemokine activation by e.g. stromal cell derived factor-1 (also known as CXCL12), MCP-1 or of neutrophil chemoattractant chemokine, CXC, motif, ligand 5 (CXCL5) has been shown to regulate myeloid cell influx. Enterocyte-derived chemokine, CXCL5 can attract chemokine, CXC, Motif, receptor 2 $(CXCR2)^+$ neutrophils into gut tissues [67]. In vivo processing of CXCL5 by MMP-2 and MMP-9 promotes neutrophil recruitment in a model of peritonitis [68]. We showed that plasmin activation enhanced the infiltration of $Gr\text{-}1^+$ neutrophils into colonic tissues in mouse models of IBD in part by increasing the biologically active neutrophil chemoattractant CXCL5 in an MMP-dependent manner [16]. It was reported that elevated CXCL5 was a significant and independent prognostic factor of survival in all colorectal cancer patients [69]. It was reported that colorectal lamina propria and submucosal regions of the colons showed CXCL5 expression, as did infiltrating neutrophils in a murine model of chronic colitis associated carcinogenesis in mice [70]. This infiltration was associated with an increase in neutrophil-associated proteases like MMP-9 and neutrophil elastase. Neutrophil depletion by antibodies in these mice prevented colitis-associated colon cancer and reduced tumor size. These studies highlight the importance of tumor-associated neutrophils. It is interesting to speculate that fibrinolytic factors regulate the influx and may even regulate the activation status of tumor-associated neutrophils.

Tumor-associated macrophages enhance tumor progression. We showed that during colitis progression plasmin enhanced macrophage infiltration in colitis tissues of IBD mice [16]. It is interesting to speculate that plasmin might be involved in colon carcinogenesis as chronic inflammation can enhance carcinogenesis by controlling inflammation and inflammatory cell influx. Popivanova et al. demonstrated that blockade of MCP-1 reduced chronic colitis-associated carcinogenesis [71]. Tumor growth is associated with macrophage density and seems to determine the speed of tumor growth. S100A10 interacts with Annexin A2 and is responsible for the conversion of PLG to plasmin by cancer cells and normal cells like macrophages [72]. It was recently shown that S100A10-deficient macrophages prevented tumor growth of murine Lewis lung carcinomas or T241 fibrosarcomas [73]. The tumor growth deficit corresponded with a decrease in macrophage density that could be rescued by intraperitoneal injection of wild-type but not S100A10-deficient macrophages

We showed that $CD11b^+F4/80^+$ cells infiltrate into T cell lymphoma tissues in a plasmin and MMP dependent manner. Using a neutralizing antibody we were able to show that $CD11b^+$ cells regulated lymphoma cell growth [14]. Plasmin-mediated myeloid cell recruitment was not associated with increased neoangiogenesis, but rather proteolytic activity was necessary for myeloid cell migration in our models of T cell lymphoma.

3. Plasmin affects tumor growth by altering myeloid cell-driven and growth factor driven neoangiogenesis

Angiogenesis, the formation of newly formed vessels is an essential step during cancer progression. The growing and metastasizing tumor needs to establish a stable blood supply to supply the tumor with nutrients and O_2. PAI-1 is one of the target genes of hypoxia inducible factor-1alpha. Fibrinolytic factors are activated in hypoxic tissues and hypoxia is the main driver of angiogenesis. Plasmin modulates angiogenesis by modifying the bioavailability of angiogenic factors, and/or changing angiogenesis-modulating leukocytes (myeloid cells). Others and we showed that plasmin activation improves myeloid cell influx into ischemic tissues in ischemic tissue regeneration models [48,49].

3.1. Plasmin can alter the bioavailability of angiogenic factor or its inhibitors

Pro- and antiangiogenic factors facilitate vascular remodeling, which results in the formation of new blood vessels [74]. Growth factor activation or their release from ECM reservoirs by proteases can propagate the angiogenic response by facilitating a sustained production of active pro- (or anti-) angiogenic factors within the tissue. Plasmin and MMPs such as MMP-2 or MMP-9 can liberate angiogenic factors such as VEGF-A and basic fibroblast growth factor (bFGF, also known as FGF-2) from immobilized matrix stores [75–77]. In particular FGF-1 and FGF-2 directly affect tumor angiogenesis by promoting endothelial cell proliferation [78,79], and have gained attention as anti-cancer targets (for review see Passaneti et al. [79]).

Because of its proteolytic activity plasmin can alter the activity of vascular endothelial growth factor-C (VEGF-C) and promote the generation of angiostatin. Angiostatin is a 38-kDa fragment of the plasminogen protein, and an endogenous inhibitor of angiogenesis [80].

VEGF-A is a, dimeric, disulfide-bound glycoprotein (34- to 42-kDa). The heparin-binding domain determines binding to the ECM. The longer forms, VEGF-A_{189} or VEGF-A_{206}, are highly basic proteins bound to extracellular heparin-containing proteoglycans. VEGF-A_{165} has intermediate properties [81]. VEGF-A_{145}, VEGF-A_{189}, and VEGF-A_{206} are bound tightly to cell surface heparin-containing proteoglycans in the ECM. VEGF-A enhances endothelial cell proliferation. VEGF increases vascular permeability that allows the extravasation of plasma proteins including plasma fibrinogen to the tumor site. Plasmin rapidly activates fibrinogen to form cross-linked fibrin [82]. Plasmin and heparin can liberate VEGF-A_{165}, VEGF-A_{189}, and VEGF-A_{206}. Cleavage of VEGF-A_{165} and VEGF-A_{189} by plasmin results in fragments containing the 110-amino terminal amino acids (VEGF-A_{110}) that are highly diffusible [83]. These events transform the microenvironment of normal tissues into a proangiogenic environment [84].

Endothelial cells of the cancer microenvironment can directly regulate tumor growth through the paracrine release of endothelial-derived growth factors and trophogens, recently termed angiocrine factors [85]. Angiocrine factors are growth factors or trophogens, adhesion molecules such as intercellular, and chemokines, like MCP1; and stromal cell derived factor-1 (SDF1; also known as CXCL12). We propose that the activation of the mainly endothelial-derived fibrinolytic factor tPA directly or indirectly via other fibrinolytic factors or proteases is the control switch of these angiocrine factors.

3.2. Plasmin recruits myeloid cells that serve as an "angiocrine factor production factory" in the microenvironment

The tumor microenvironment harbors both tumor suppressive and tumor supportive (proangiogenic) myeloid cells (for detailed review

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[86–88]). In contrast, various other myeloid subpopulations, such as Tie2 expressing monocytes, neutrophils, eosinophils, mast cells, and dendritic cells, have been shown to actively participate in tumor angiogenesis and progression.

What are these myeloid cells and where do they come from? Monocytes, macrophages and granulocytes/neutrophils belong to the myeloid cell lineage through differentiation from myeloid progenitor cells or the most primitive hematopoietic stem cells. These precursors or stem cells reside in the bone marrow. Hematopoietic growth factors like G-CSF, granulocyte-macrophage colony-stimulating factor and KitL promote the maturation into terminally differentiated neutrophils or monocytes. We demonstrated that plasmin expands myeloid cells within the BM by MMP-9-mediated KitL release [26,36]. Similarly, angiogenic factor elevation (angiopoietin-1 and VEGF-A_{165} or placental growth factor) enhances cell differentiation into the myeloid lineage [89,90]. After angiogenic factor elevation, higher numbers of myeloid monocytes and myeloid progenitors were found in circulation.

When blood-circulating monocytes migrate into tissues (incl. cancer tissues), they differentiate into resident tissue macrophages. Macrophage diversity enhances tumor progression and metastasis (for review see Qian et al. [91]). Dependent on the cytokine microenvironment M1 (classically activated) and M2 (alternatively activated) macrophages can be identified. A recent study demonstrated that tPA promotes M1 macrophage survival through p90RSK and p38 mitogen activated protein kinase pathway [92].

The fibrinolytic pathway can control myeloid cell influx and neovascularization, a key mechanism for tumor progression. MMP-1, -3, -9, -12, and urokinase are important proteases that degrade the ECM and release other growth factors to stimulate angiogenesis. A study has shown that targeting MMP-9 expressing microphages prevented cervical carcinogenesis and tumor angiogenesis [93]. It was shown that VEGF-A released from tumor cells drives the proliferation and myeloid differentiation of hematopoietic stem cells into the granulocyte-1 (Gr-1)/Mac-1/CD31 subpopulation [94].

We reported that a serpin-resistant form of tPA by activating the extracellular proteases MMP-9 and plasmin expands the myeloid cell pool and mobilizes $CD45^+CD11b^+$ proangiogenic, myeloid cells by activating the extracellular proteases MMP-9 and plasmin [48]. tPA improved the incorporation of $CD11b^+$ cells into ischemic tissues and increased the expression of neoangiogenesis-related genes, including VEGF-A. Remarkably, transplantation of BM-derived tPA-mobilized $CD11b^+$ cells and VEGFR-1^+ cells, but not carrier-mobilized cells or $CD11b^-$ cells, accelerated neovascularization and ischemic tissue regeneration. Inhibition of VEGF signaling suppresses tPA-induced neovascularization in a model of hind-limb ischemia. Thus, tPA mobilized $CD11b^+$ cells from the BM and increased systemic and local (cellular) VEGF-A, which can locally promote angiogenesis during ischemic recovery. Other groups showed that tPA increases F4/80 macrophage accumulation in an acute brain injury model [95]. In models of acute and chronic injury, tPA promoted infiltration of macrophages or other leukocytes [96,97].

Similar pharmacologic inhibition of PAI-1 promoted angiogenesis and prevented tissue necrosis in a mouse model of hind-limb ischemia [49]. PAI-1 inhibitor treatment augmented FGF-2 and FGF-R1 expression. FGF-2-induced angiogenesis requires VEGF signaling [98]. Inhibition of VEGF-A and FGF-2 signaling with antibodies against murine VEGF-A and FGF-2 blocked the PAI-1 inhibitor-mediated FGF-2 and FGFR-1 increase in VWF^+ cells. These data indicate that the proangiogenic effects observed after PAI-1 inhibitor treatment are mediated by the proangiogenic factors FGF-2 and VEGF-A, and that neutrophils seem to be a source for these growth factors. During this process, improved tissue regeneration was due to an expansion of circulating and tissue-resident Gr-1^+ neutrophils and to increased release of the angiogenic factor VEGF-A, the hematopoietic growth factor KitL, and G-CSF.

4. Future directions

The microenvironment is capable of controlling tumor growth. Reeducation of microenvironmental cells (e.g. endothelial or stromal cells), rather than targeted ablation may be an effective strategy for treating cancer. Proteases are often highly expressed in cells of the microenvironment. We propose that proteases alter the cellular performance of microenvironmental cells by generating important substrates and regulating the cellular influx at the tumor-stroma interface. Strategies to selectively block proteolytic activity in microenvironmental cells using cell-specific antigens and receptors attached to a macromolecular carrier (tissue specific drug delivery) will avoid side effects associated with systemic blockade of proteases, and allow for in-situ drug accumulation of the drug. We suggest that normalization of the proteolytic activity in microenvironmental cells might be a novel strategy to control tumor growth by reeducating its environment.

Myelosuppressive drugs, included in virtually all cancer treatment regimens can activate proteases within the microenvironment, creating a proteolytic niche that protects drug-resistant cancer cells [2,36,99]. We discussed in this review how the fibrinolytic pathway in the growing tumor builds the proinflammatory environment by enhancing myeloid cell generation, infiltration and cytokine release. Myeloid cells facilitate tumor angiogenesis and contribute to refractoriness to antiangiogenic therapy [100]. FGF-2, which is activated by plasmin, seems to play an integral role in the resistance to antiangiogenic therapy, and agents that specifically target the FGF pathway are being developed and tested in clinical trials [101]. Therefore understanding how myeloid cells are recruited is of critical importance in the treatment of cancer. In certain tumors, blockade of the recruitment of BM-derived hematopoietic cells prevented tumor growth and angiogenesis [102].

We propose that drugs targeting fibrinolytic factors in the cancer microenvironment together with drugs targeting the tumor cells might ensure better cancer growth control. We suggest that local regulation rather than systemic inhibition will be a novel anti-cancer strategy.

Conflict of interest

The authors declare that there is no conflict of interest.

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