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REVIEW

Pathologies at the nexus of blood coagulation and inflammation: thrombin in hemostasis, cancer, and beyond

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Abstract Thrombin is the protease involved in blood coagulation. Its deregulation can lead to hemostatic abnormalities, which range from subtle subclinical to serious life-threatening coagulopathies, i.e., during septicemia. Additionally, thrombin plays important roles in many (patho)physiological conditions that reach far beyond its well-established role in stemming blood loss and thrombosis, including embryonic development and angiogenesis but also extending to inflammatory processes, complement activation, and even tumor biology. In this review, we will address thrombin's broad roles in diverse (patho)physiological processes in an integrative way. We will also discuss thrombin as an emerging major target for novel therapies.

Keywords Coagulation · Inflammation · Hemostasis · Cancer · Thrombin (F2) · Thrombosis · Immune system · Metastasis · Angiogenesis · Invasion · Prognosis · Trousseau's syndrome

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Introduction

The serine protease thrombin: from blood coagulation to far beyond

Thrombin is the key effector protease of the blood coagulation system. Although it is best known for this role, it directly contributes to other processes including embryonic development, angiogenesis, organ regeneration [1, 2], innate immunity, acute and chronic inflammatory processes [3], atherosclerosis [4], neuropathology [5], and tumor biology [6, 7] (synopsis, Fig. 1).

The wide spectrum of thrombin functions and its role in physiology and pathophysiology are generally explained by its activity as a serine protease (acting both on soluble and membrane-bound substrates, Table 1). To promote blood coagulation, thrombin converts circulating fibrinogen into fibrin, but it can also serve as a signaling molecule to cells through protease-activated receptors (PARs, Fig. 2) [11]. PARs are G protein-coupled receptors that carry their own ligands, which remain cryptic until unmasked by receptor cleavage. Upon binding of thrombin, an extracellular proteolytic cleavage event is converted into a transmembrane signal, a principle that can account for the vast majority of known thrombin functions on cells.

When acting on cells, thrombin triggers a wide spectrum of responses such as cell proliferation, cell division, and changes of the cell morphology and motility. It induces downstream signal transduction cascades thereby affecting electrophysiology, metabolic processes, and global gene expression. In addition, thrombin has a crucial function during acute and chronic inflammatory processes, e.g., by activating the complement cascade, or as a mitogen for immune effector cells [3, 11] (further detailed below). Thrombin also plays an important role for the initiation, formation, and propagation of atherogenesis and thereby

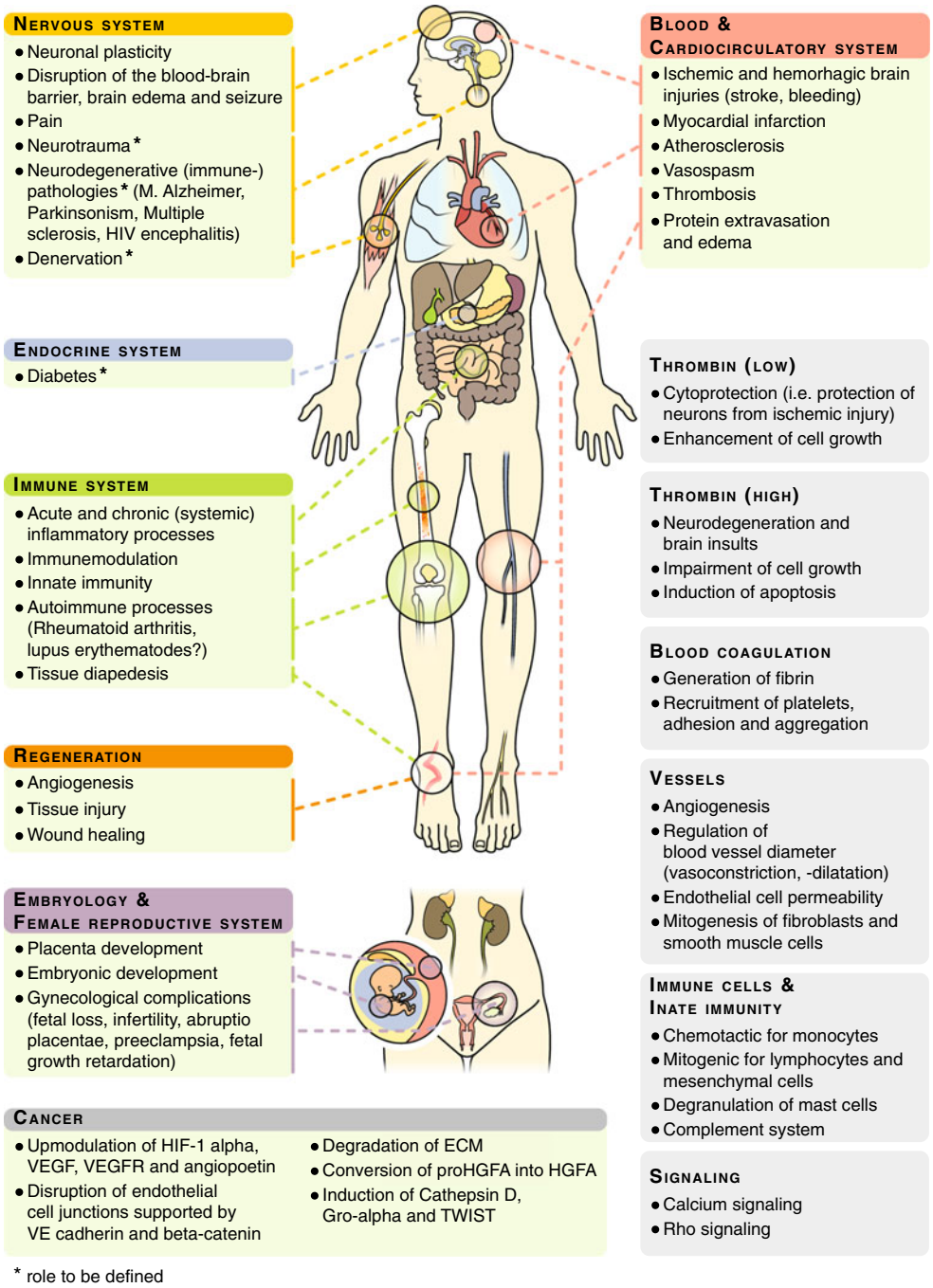

Fig. 1 Thrombin and thrombin functions in development, physiology, and pathophysiology

collectively illustrates the whole plethora and wide (patho)physiological relevance in many systems including hemostasis, inflammation, proliferation, and vasomotor regulation. Thrombin, however, also induces tumor growth, metastasis, and angiogenesis and might serve to preserve dormant tumor cells in individuals, preventing host eradication [6]. Thus, thrombin plays an important role in an unforeseen dimension of various (patho)physiological processes with significant incidence, prevalence, and mortality—apart from the well-established role in stemming blood loss and its perturbances.

More general information concerning thrombin and its role in blood coagulation is provided in earlier reviews [12–15]. Here, we will focus on some of the most important (patho)physiological processes mediated by thrombin and illustrate how recently uncovered regulatory mechanisms

Table 1 Thrombin actions and its regulation in the hemostatic system

Thrombin function in hemostasis	Natural inhibitors of thrombin
Procoagulant • Conversion of fibrinogen to fibrin • Activation of F XIII • Activation of F VII • Activation of F Activation of F VIII • Activation of F V • Activation of thrombocytes (via PARs)	Antithrombin (AT III, SERPINC1) Plasma inhibitor, produced in the liver, causes proteolytic inactivation of thrombin, and activates fibrinolysis (via induction of the tissue type plasminogen activator in endothelial cells). Most important physiological inhibitor of the coagulation system (inhibits also activated F IX, X, XI, and XII), anticoagulant activity of AT III is dependent on its cofactor, heparin [8].
Anticoagulant • Binding to thrombomodulin (TM) and activation of protein C	Thrombomodulin (TM, THBD) Integral membrane-bound protein expressed on endothelial cells, thrombin binding turns it into an anticoagulant enzyme (by activation of the protein C pathway, Fig. 2) [9].
Antifibrinolytic • Binding to thrombomodulin (TM) and activation of TAFI (Thrombin-activatable fibrinolysis inhibitor).	Heparin cofactor (HCII, SERPIND1) Second plasma inhibitor, shares homology with ATIII. Produced in the liver, it specifically inhibits thrombin in the presence of many polyanionic molecules including glycosaminoglycans, heparin, or dermatan sulfate (the latter of which originates from intact endothelial cells), contributes 20–30 % to thrombin inhibition in blood coagulation [8].
	Alpha-2-Macroglobulin (A2M) Nonspecific plasma serine protease inhibitor, produced in the liver. Inhibits coagulation by inactivating thrombin and kallikrein, proposed to act as a backup inhibitor of thrombin in adults, represents an important progressive inhibitor of thrombin in young infants [10].
	Alpha-1-Antitrypsin (SERPINA1) Nonspecific plasma serine protease inhibitor, produced in the liver. Inhibits primarily activated F XI, but also thrombin, kallikrein, plasmin, and the tissue type plasminogen activator [8].
	Protein C inhibitor (PCI, SERPINA5) Nonspecific plasma serine protease inhibitor, produced in the liver. In the presence of heparin, PCI is anticoagulant, inhibiting proteolytic cleavage of fibrinogen by thrombin. In the presence of TM, PCI is procoagulant, inhibiting the activation of protein C by thrombin [8].
	Plasminogen activator inhibitor-1 (PAI-1, SERPINE1) Nonspecific serine protease inhibitor, synthesized in endothelial cells, platelets, and other mesenchymal cells surrounding the vasculature. Acts primarily to inhibit premature fibrinolysis after release from activated platelets and disintegrating endothelia; inhibits also thrombin and APC in the presence of vibronectin and/or heparin, contribution to blood coagulation in this context unknown [8].

governing thrombin gene expression might explain previously enigmatic links between blood coagulation and cancer.

Thrombin in adaptive and innate immunity

Thrombin is perhaps the most effective agonist for platelet activation; upon binding to PARs, it triggers a shape change of platelets and the release of the platelet activators ADP, serotonin, thromboxane, as well as a variety of chemokines and growth factors. Furthermore, it liberates the major fibrinogen receptor GPIIb-IIIa integrin complex and P-selectin, and mobilizes the CD40 ligand to the platelet surface. While the first two enhance platelet aggregation [16], CD40L induces endothelial cells to secrete chemokines and to express adhesion molecules, thereby generating signals for the recruitment and extravasation of leukocytes [17]. Thrombin also elicits responses in the vascular endothelium including shape and permeability changes, mobilization of adhesive molecules such as vWF and P-selectin, and the production of various cytokines.

Crucially, PAR expression is found on many immune cells, including macrophages, monocytes, dendritic cells, lymphocytes, and mast cells. Thus, is it not surprising that thrombin influences many cellular functions with important roles in immunity; thrombin is chemotactic for monocytes, regulates

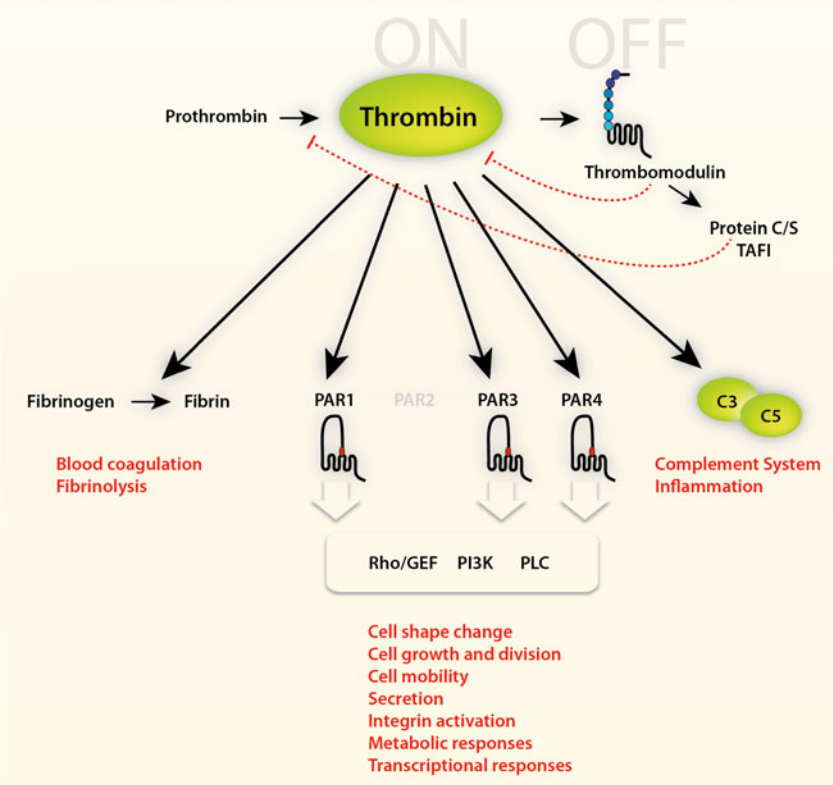

Fig. 2 Mechanisms of thrombin action. Thrombin is a multifunctional serine protease involved in blood coagulation, complement activation, and numerous cellular functions mediated via G protein-coupled prote'ase-activated receptor (*PAR*) signaling pathways (for further details, see [11]). Thrombin is antagonized by binding to thrombomodulin, a multi-domain proteoglycan found primarily on endothelial cells (see natural inhibitors of thrombin, Table 1). However, thrombin bound to thrombomodulin augments the ability to activate protein C, a natural anticoagulant, which in a negative feedback loop represses the generation of thrombin (protein C itself also has antiapoptotic and anti-inflammatory activity and increases activation of thrombin-activatable fibrinolysis inhibitor (*TAFI*), an enzyme which blocks the activation of plasminogen and inactivates vasoactive peptides like complement C5a, not shown)

cytokine production in fibroblasts, and is mitogenic for lymphocytes and mesenchymal cells [11]. However, it also regulates a plethora of further responses in immune cells via activation of PAR signaling [18, 19]. In the complement system, activated thrombin can directly generate C5a and thereby bypass the classical, the alternative, and the lectin pathway to trigger complement activation [20]. Yet, this is not limited to thrombin—almost all serine proteases in the coagulation system signal into the complement system, and vice versa, as descendants of a common ancestral pathway, proteolytic components from the complement system feed into the blood coagulation system [3] (Fig. 3). Thus, thrombin belongs to a complex network in which mutual connections between these two pathways dictate the activity of the entire "coagulo-complementome". Finally, thrombin promotes the activation of various pro-inflammatory pathways including the production of pro-inflammatory cytokines (such as TNF, IL-1β, and IL-6)—and cytokines, in turn, can stimulate coagulation [3, 20–22].

In summary, apart from generating fibrin to promote hemostasis, thrombin has a host of direct actions on different cell types including platelets and endothelial cells and also various effector cells of the immune system. Moreover, thrombin belongs to the "plasma serine protease system" in which the coagulation and complement systems are tightly connected through multiple direct interactions of serine proteases. In the setting of sepsis, for instance, the extensive cross talk between the coagulation pathways and the complement system is of particular importance, as their uncontrolled activation essentially contributes to and further perpetuates the detrimental pathogenesis of the disease (see for further details [3]).

Thrombin in acute and chronic inflammatory processes

As thrombin acts both on cellular and soluble effectors of the immune system, it has pivotal roles in acute and chronic inflammatory processes [23, 24, 166], many of which are regulated by activation of PARs on respective effector cells [18, 19]. Thus, apart from physiological wound healing where thrombin helps attracting effector cells to organize and repair damaged tissues [25], overwhelming inflammatory responses

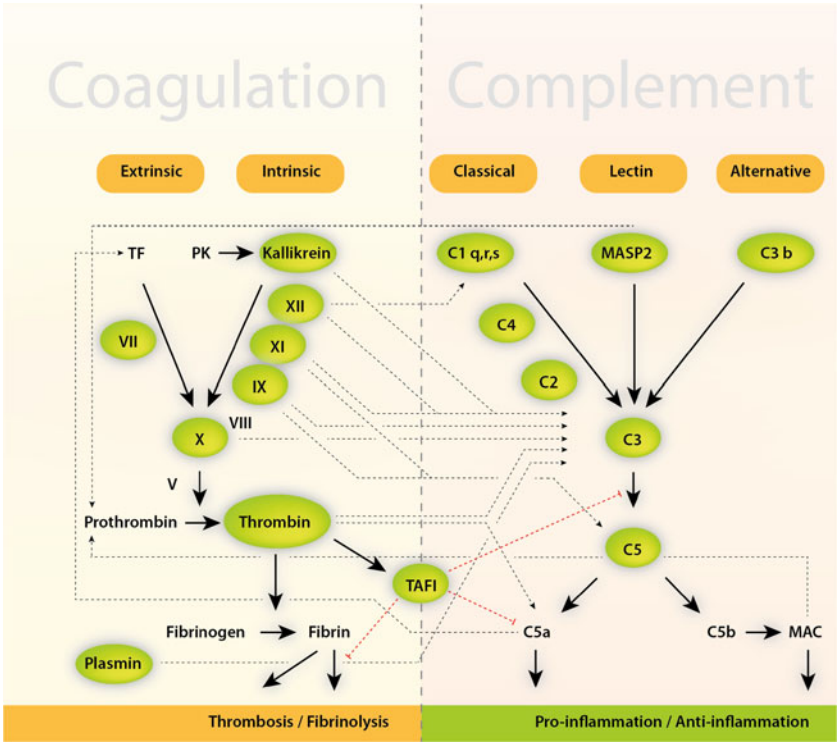

Fig. 3 Cross talk between the coagulation and complement system. The coagulation cascade, the complement system, and fibrinolysis (simplified) communicate through many direct and bidirectional interactions (indicated). Activated clotting Factor XII can activate the classical complement pathway through cleavage of the complement component *C1*. Similarly, *thrombin*, *kallikrein*, and *plasmin* directly cleave complement component *C3*, as well as its activation fragments (not shown). Moreover, thrombin can cleave *C5* into *C5a*, which occurs independently of *C3* and therefore represents a bypass of the three traditional complement activation pathways (the classical, the lectin, and alternative pathways) [3]. Thrombin-activatable fibrinolysis inhibitor (*TAFI*) inactivates *C3a* and *C5a* in a negative feedback loop. The complement system also amplifies coagulation through the C5a-mediated induction of expression of tissue factor (*TF*) and plasminogen activator inhibitor 1 by leukocytes (not shown), the latter of which inhibits fibrinolysis. In addition, mannan-binding lectin serine protease 2 (*MASP2*) of the lectin complement activation pathway triggers coagulation by converting prothrombin to thrombin. *MAC*, membrane attack complex (C5b–C9); see also [3, 161]

triggered by thrombin can also cause detrimental responses involved in the pathophysiology of rheumatoid arthritis [26]. Accordingly, thrombin inhibition down-modulates synovial inflammation and has been shown to ameliorate even established arthritis [27]. Interestingly, in the pathogenesis of arthritis, thrombin activation (induced by collagen) exerts a dual function: it leads to an increased expression of PAR-1 in the inflamed joint and it serves, at the same time, as "ligand" for PAR-1-mediated activation of synovial hyperproliferation and an inflammatory destruction [28, 29]. Furthermore, thrombin is implicated in the pathogenesis of inflammatory brain diseases such as multiple sclerosis [30] and possibly Alzheimer's disease [31–33]. In both cases, uncontrolled inflammatory processes that are triggered by thrombin (predominantly via activation of PARs) are suspected to contribute to the progression of inflammatory brain diseases and neuronal tissue damage by NMDA receptor response potentiation, apoptosis, and inappropriate glial proliferation. Aberrant induction of thrombin can also compromise neuronal function by disturbing the electrophysiology resulting in conduction blocks or seizures [30, 34]. Despite its detrimental role also in edema formation (as a result of thrombin-mediated permeability changes of endothelia) following intracerebral hemorrhage [35] or in vascular dementia [36] and memory impairment [37], thrombin has a neuroprotective function, especially at lower concentrations [38, 39]. Although cell death and a protective function of thrombin share initial signaling components, differences in the amplitude as well as the duration of the signal may result in different final pathways thus explaining the functional dichotomy of thrombin at different concentrations. Finally, rather reflecting its direct function in blood coagulation, the generation of thrombin resulting in local thrombosis and/or fibrin deposition limits the survival and dissemination of some microbial pathogens (by generating a "mechanical" barrier) and might thereby affect host susceptibility to a variety of infectious diseases [40, 41] (for review, [167]). However, although thrombin is also known to be upregulated in various chronic disease entities, the underlying mechanism and exact pathogenetic relevance, whether or not thrombin functions as a driver or passenger in these disease processes, is still poorly understood [31, 32, 42]. Yet, it needs to be noted that (selective) PAR-1 expression and activation, i.e., on dendritic

cells, play a critical role in both chronic and acute lethal inflammatory processes [43, 44], putting blood coagulation (and thrombin specifically) also on center stage for an active immune modulatory cellular function.

Thrombin and atherosclerosis

Thrombin is generated at the site of vascular injury and has been proposed to play a crucial role in the pathogenesis of atherosclerosis by activating platelets and promoting a pro-inflammatory response [4, 25, 45]. This is characterized by an increased production of diverse chemokines and cytokines, cell adhesion molecules, enhanced vascular permeability, migration and proliferation of vascular smooth muscle cells, wall thickening, and vasoconstriction. Thus, thrombin is considered to contribute to both the initiation and also the propagation of atherosclerotic lesions. This eventually results in a vicious circle, where progressing endothelial injuries cause further thrombin conversion with detrimental self-sustaining qualities. These findings are further corroborated by mouse models, where the deletion of the natural thrombin inhibitor (heparin cofactor II, see Table 1) promotes an accelerated atherogenic state. In contrast, reduction of thrombin activity attenuates plaque progression and promotes stability in advanced atherosclerotic lesions [46]. Thus, with the advent of novel selective anticoagulants such as direct thrombin inhibitors [47] or PAR inhibition [48], great hope accompanies basic research to find potentially new therapeutic strategies to interfere with thrombin's role in atherosclerosis. In preclinical models, selective PAR-1 blockade resulted in potent inhibition of thrombin-induced platelet aggregation but appeared to preserve primary hemostatic function [49]. These findings clearly put selective PAR-1 inhibition on center stage as a promising target to interfere with atherosclerosis. Interestingly, while PAR-1 inhibition reduces the risk of cardiovascular death or ischemic events with stable atherosclerosis [50], such effects have not been witnessed so far for acute coronary syndromes [51]. Both studies revealed that PAR-1 inhibition leads to an increased risk of moderate and severe bleeding, including intracranial hemorrhage, thus highlighting the need to optimize the therapeutic regimen to specifically interfere with thrombin's contribution to the initiation, formation, progression, and destabilization of atherosclerotic plaques. Possibly, key to that might be that thrombin itself has earlier been observed to be regulated in response to (chronic) inflammatory events [42] (further detailed below).

Thrombin in embryonic development and angiogenesis

One of the most striking observations regarding "non-classical" thrombin functions has been made in knockout animals: Predictably, a functional null allele results in severe coagulation abnormalities leading to embryonic and neonatal lethality [52, 53]. However, thrombin is also implicated in maintaining vascular integrity during development as well as postnatal life [41, 52, 53], which is mainly driven by the activation of PARs [11]. Interestingly, thrombin expression at levels of as little as 5–10 % of the norm is still compatible with normal embryonic development, but the resulting animals are hemophilic without showing spontaneous bleeding [54]. This indicates that low levels of thrombin expression are indispensible for normal embryonic development and higher levels are required to control bleeding. Ultimately, thrombin is highly expressed and extensively regulated in muscles during (neonatal) synapse remodeling [55], after muscle denervation, and during brain development [56, 57], suggesting a role of thrombin in neuronal plasticity. This is highlighted by findings of thrombin being produced in the brain either immediately after cerebral hemorrhage or after breakdown of the blood–brain barrier, which occurs in response to many kinds of brain injury [58]. Furthermore, transient global ischemia up-modulates thrombin gene expression in the brain [59].

Thrombin in tissue and organ regeneration and differentiation

The importance of thrombin in tissue regeneration is highlighted by studies of the vertebrate lens [1, 2]. Here, selective thrombin activation has been discovered to control the cell cycle reentry at the site of tissue injury and thereby initiates the process of vertebrate lens regeneration. Thrombin also counteracts the postmitotic arrest in newt myotubes and thereby plays an important role in plasticity and reprogramming of differentiated cells in amphibian regeneration [60]. Yet, also in humans, it regulates (hematopoietic) stem and progenitor cell functions [61], it stimulates various differentiation processes [62, 63], and it has been reported to be up-modulated after spinal cord injuries [64] and other neurotraumas [56]. On the other hand, elevated thrombin production is associated with aging [65] and has been reported to contribute to the development of age-related (neuronal) deficits [37] or an increased propensity for developing blood clots at old age [65].

Thrombin in cancer and tumor biology

Blood coagulation factors in general and thrombin in particular have recently been found to play an important role in cancer biology [6, 7, 66–70]: In tumor patients, increased procoagulatory activities are almost inevitably seen at some point during tumor progression, where tumor procoagulants are released into the blood stream and thus give rise to the development of thrombosis with serious—often life-threatening—

consequences. However, thrombosis can also represent a forewarning of an as yet undiagnosed "occult" malignancy (so called Trousseau's syndrome) [71]. Thus, hypercoagulabilities have not only serious therapeutic but also important diagnostic implications. Although considered to be the consequence of an underlying tumor disease for almost 150 years, recent evidence suggests that this syndrome is not a mere paraneoplastic effect, but the result of mechanisms that provide a selective advantage to cancer cells [72–77]—with a striking impact on tumor initiation, tumor progression, and patient prognosis [72, 74, 75, 77–79]. In line with these findings, hyperactivation of blood coagulation is associated with more rapid tumor progression [72–74]. Conversely, impaired blood coagulation reduces the incidence of cancer [75] and inhibits the invasive growth of tumor cells and metastasis in patients treated with anticoagulants [77, 78] or in mice defective for coagulation factors such as fibrinogen [80] or thrombin [81].

How does thrombin influence tumor biology?

In 1986, Harold Dvorak described parallels between wound healing and tumor disease, where hemostasis (local fibrin deposition) is an inherent part of physiological regeneration processes, which are also engaged during tumorigenesis [82]. This includes almost all factors of primary and secondary hemostasis. It involves the direct activation of thrombin and fibrin synthesis by production of pro-coagulatory substances by tumor cells and/or indirectly via the activation of endothelial cells, thrombocytes, and leukocytes by production of cytokines, proteases, glycoproteins, and tissue factor-loaded microparticles [83]. Eventually, this creates a protumorigenic micromilieu, which drives cellular programs promoting cell growth, motility, angiogenesis, and invasiveness [7].

Tissue factor is one of the most important tumor-associated determinants for tumor progression and metastasis (i.e., by the induction of tissue factor signaling promoting tumor growth and angiogenesis; see [84, 85] for review). In addition, tissue factor mediates (local) thrombin generation (Fig. 3), which is crucial for various protumorigenic processes [6, 70, 86, 87]. The critical role of thrombin in augmenting protumorigenic cellular programs reflects the whole plethora of thrombin functions—including its dual role in fibrin formation and platelet activation, the activation of PAR signaling, the proteolytic breakdown of extracellular matrix, and/or direct oncogenic mechanisms (i.e., via induction of c-myc or co-activation of the hepatocyte growth factor) [6, 69, 84, 88–90]. Specifically, thrombin generation is crucial for metastasis not only through fibrin and platelet deposition but also via thrombin-dependent PAR-1 signaling [86, 91–95]. Thrombin stimulates tumor adhesion [91, 92, 94, 96, 97], growth [98], DNA synthesis, and cellular proliferation either directly or in synergy with other mitogens [86, 99]. Thrombin is an effective activator of angiogenesis by clotting-dependent mechanisms involving platelet activation and fibrin deposition. However, thrombin also induces tumor angiogenesis via clotting-independent mechanisms mediated by PAR activation, which leads to an upregulation of various growth factors, including VEGF [100], angiopoetin-1 [101] and angiopoietin-2 [102], the major VEGF receptor KDR, as well as MMP1 and MMP2 in endothelial cells [103, 104]. Furthermore, activated platelets augment the pro-angiogenic process by releasing VEGF and platelet-derived growth factors [100, 105]. Finally, thrombin-dependent fibrin formation and platelet activation create a niche protecting the emerging tumor against natural killer cell attacks [106–108].

In patients, the appropriate control of thrombin expression and activation therefore not only determines the delicate balance of pro- and anticoagulatory activities [109–112] but also effects tumor dissemination and metastasis [81, 113]. Accordingly, the prevalence of mutations increasing thrombin expression (such as F2 20210 G>A) are higher in some cancer patients compared to controls [76], and tumor cells treated with thrombin display increased metastatic potential [81, 91]. These observations are highlighted by our own data showing that thrombin gene expression is specifically upregulated in metastatic prostate and colon cancer (Fig. 4; [113]), which ultimately leads to PAR activation and induction of genes involved in thrombin-mediated invasion and angiogenesis.

Inversely, low-level thrombin expression [81] or the specific inhibition of thrombin by sulfohirudin or thrombostatin reduces tumor growth and metastasis in vivo [114, 115]. This prometastatic function of thrombin is further corroborated by findings demonstrating that the endogenous generation and/or activity of thrombin—by altering the thrombomodulin system—plays a crucial role for spontaneous metastasis in vivo [116]. Finally, the expression of thrombin-activated receptors (PAR-1) is frequently up-modulated in highly metastatic tumors [88], which correlates with negative prognosis [117]. Interestingly, numerous reports document beneficial effects of pharmaceutical thrombin inhibition for cancer patient survival (for reviews, see [6, 70, 118] and special issue, Journal of Clinical Oncology [77, 83]).

In summary, thrombin contributes to various hallmark processes directly associated with tumor dissemination and progression including (1) cellular proliferation and tumor growth, (2) tumor adhesion to subendothelial matrix, (3) tissue invasion and extravasation, (4) tumor-associated angiogenesis, (5) tumor-associated pro-inflammatory processes, and (6) the colonization of a metastatic niche (fibrin net encapsulation and platelet activation; Table 2).

Whether thrombin also plays a role for tumor initiation and early events in tumorigenesis is yet to be determined. Nevertheless, it is noteworthy that the prevalence of prothrombotic mutations increasing thrombin gene expression is higher in

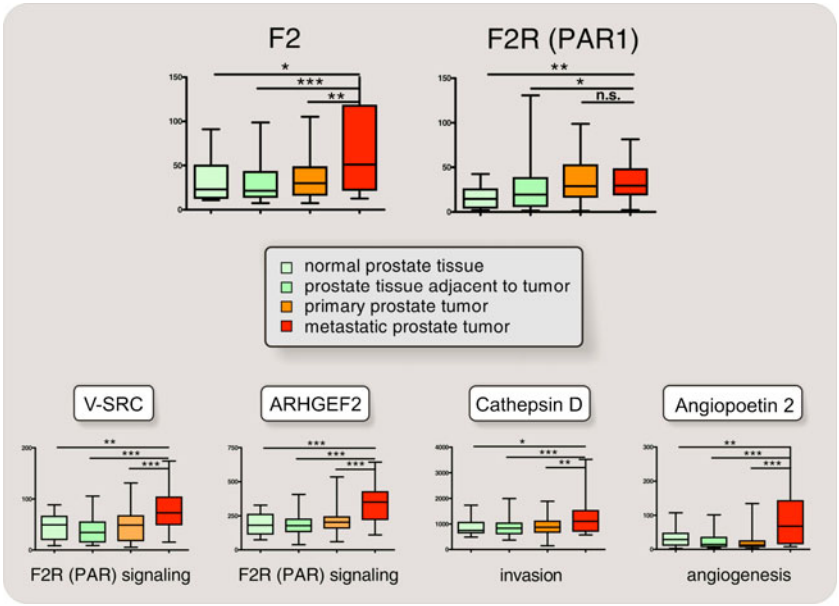

Fig. 4 Stage-dependent induction of ectopic (i.e., extrahepatic) thrombin (*F2*) gene expression in metastatic prostate cancer. Normalized mRNA expression of the thrombin (*F2*) gene, of the F2 Receptor (*F2R/PAR1*), of *V-SRC* and *ARHGEF2* (surrogate for activated F2R signaling), and of *cathepsin D* and *angiopoetin 2* (for invasion and angiogenesis [6, 137]), obtained from gene expression profiling after extraction, normalization, and reassembly of 171 human samples (see [162] GEO GDS2545 record) including metastatic prostate cancer tissues (n=24; GSE6605), nonmetastatic primary prostate tumors (n=60; GSE6606), prostate tissues adjacent to the tumor (n=63; GSE6608), and normal donor prostate tissues (n=18, GSE6604) [163, 164] (median, *horizontal line*; 25th through 75th percentile, *box*; range, standard error of the mean (SEM); *p<0.05; **p<0.01; ***p<0.001)

some cohorts of cancer patients compared to controls [76]. This might either reflect a nonfunctional association or display a potential selective disadvantage and predisposition for carriers of F2 20210 G>A for developing specific cancer entities—although this association clearly does not apply to all tumor types [74, 79].

The functional role of thrombin for tumor initiation might therefore differ with regard to specific tumor entities.

Table 2 Thrombin actions during tumorigenesis

Thrombin function	Refs.
Tumor adhesion	[91, 92, 94, 96, 97, 119]
Tumor growth	[67, 98]
DNA synthesis, antiapoptotic	[120]
Proliferation, cell cycle	[67, 121–127]
Mitogenic for mesenchymal tissues, fibroblasts, endothelial and muscle cells	[121–123]
Motility	[121, 128]
Disruption of endothelial cell junctions	[129]
Invasion	[130, 131]
Metastasis	[81, 91–95, 132]
Angiogenesis	[100–104, 133–139]
Preservation of dormant tumor cells preventing host eradication	[6, 106, 107]

Therapeutic approaches targeting thrombin may thus help to interfere with its protumorigenic properties during tumor progression, but possibly also in tumor initiation. Although several hallmark studies document the detrimental effect of disordered hemostasis for cancer onset, these observations have so far eluded mechanistic explanation [72–76, 80, 140]. A possible key to that might be that thrombin is upregulated during inflammatory events [20, 32, 42, 113, 141]. Understanding potentially underlying mechanisms could therefore help to disentangle the enigmatic relationship between blood coagulation and cancer biology, and potentially contribute to the development of novel therapeutic strategies.

When and where is thrombin generated? A p38 MAPK dependent switch controls F2 expression

In order to become biologically active, prothrombin is cleaved into thrombin. This proteolytic activation step is catalyzed by activated Factor X (Fig. 3) and controlled by various negative feedback mechanisms to prevent overwhelming pro-coagulatory activities or other uncontrolled thrombin-dependent activities (see natural thrombin inhibitors, Table 1). Nevertheless, mutations that merely increase thrombin expression (such as F2 20210 G>A) already shift the well-balanced equilibrium of pro- and anticoagulatory activities [109, 142, 143]. This

indicates that the activation step of prothrombin cleavage becomes secondary. As a consequence, prothrombin gene expression needs to be tightly controlled: even subtle changes (1.5- to 1.7-fold) of its gene expression [143, 144] can result in a clinically relevant thrombophilia [13, 145].

Although primarily synthesized in hepatocytes (in adults), thrombin is also expressed in the brain [57] and is induced in neurons after cerebral ischemia [59], during embryonic development [52, 53, 55, 57], and in various acute and chronic inflammatory processes [20, 32, 42, 56, 64, 141, 146]. Hence, cells must have evolved mechanism(s) that fine-tune thrombin expression and thereby ensure proper execution of thrombin-mediated cellular programs. Yet—as highlighted above—the underlying molecular principles governing thrombin expression regulation have remained enigmatic until recently.

Inflammatory processes represent possible triggers to induce thrombin gene expression [20, 32, 42, 113, 141, 147, 148]. In addition, the crucial role of thrombin for angiogenesis [11] suggests that a regulatory mechanism controlling thrombin expression might have evolved a sensor for low oxygen pressure. This could explain why thrombin is hyperexpressed in response to ischemic events [59] or in the tumor micromilieu of growing tumors [113], which notoriously suffer from low oxygen pressure.

We recently discovered a gene regulatory mechanism affecting RNA 3′ end processing that operates in response to and integrates environmental stimuli to fine-tune thrombin mRNA expression via p38 MAPK activation [113] (Fig. 5). In turn, p38 MAPK up-modulates the RNA 3′ end processing machinery and directly induces the phosphorylation of inhibitory proteins, which bind to a highly conserved sequence motif in the F2 3′UTR. Upon phosphorylation, these inhibitory proteins fail to bind the prothrombin mRNA, making it accessible to proteins that stimulate 3′ end processing (many of which play important roles in cancer [149]). This eventually results in a higher (pro)thrombin expression under inflammatory conditions such as septicemia. Interestingly, p38 MAPK activation also directs thrombin overexpression in the tumor microenvironment of metastasized colon carcinoma. This, in turn, activates PARs, which induces the expression of genes with crucial roles in neoangiogenesis and tumor dissemination [113] (Figs. 4 and 5), highlighting the physiological importance of novel layers of gene expression control [150–153].

The identification of p38 MAPK controlling thrombin represents a hypothetically interesting “building block” for a model, whereby (tumor-associated) hypoxia and/or ischemia might trigger thrombin expression: hypoxia represents a potent inducer of p38 MAPK [154], and its ablation results in

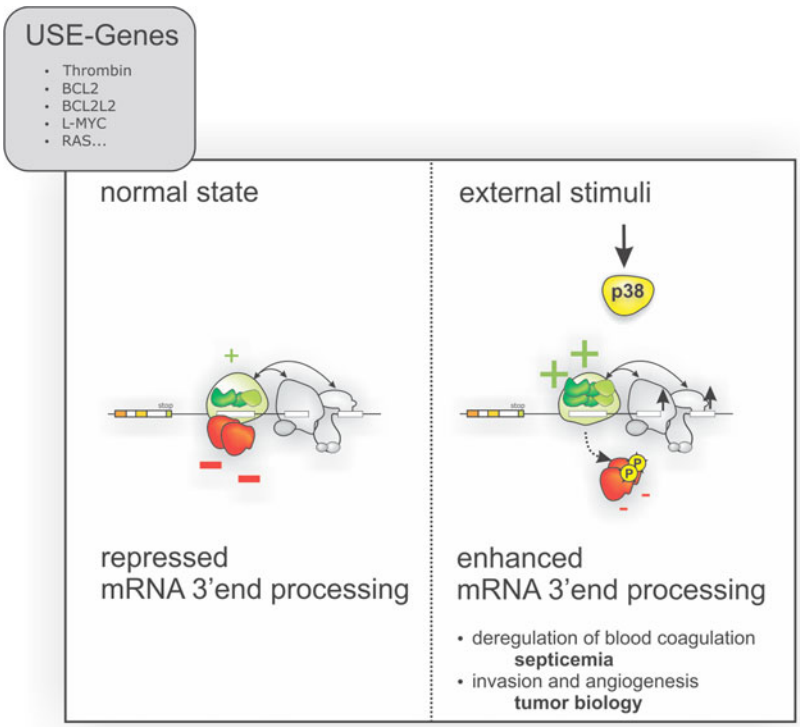

Fig. 5 Extracellular stimuli induce thrombin gene expression by p38 MAPK activation. Extracellular stimuli activate p38 MAPK and thereby phosphorylate regulatory proteins (*red*), which “catalyzes” the remodeling of a stimulatory ribonucleoprotein (RNP) complexes (*green*) to up-modulate the efficiency of thrombin mRNA 3′ end processing. This mechanism allows modulating cellular functions, such as blood coagulation by controlling the amount of thrombin protein produced. Yet activation of this mechanism also appears to play an important role in other pathophysiological processes (such as tumorigenesis) and drives cellular programs involved in tumor invasion and neoangiogenesis by the activation of thrombin receptor signaling (F2R, PAR-1) and degradation of extracellular matrix (figure adopted from Cell Press [113])

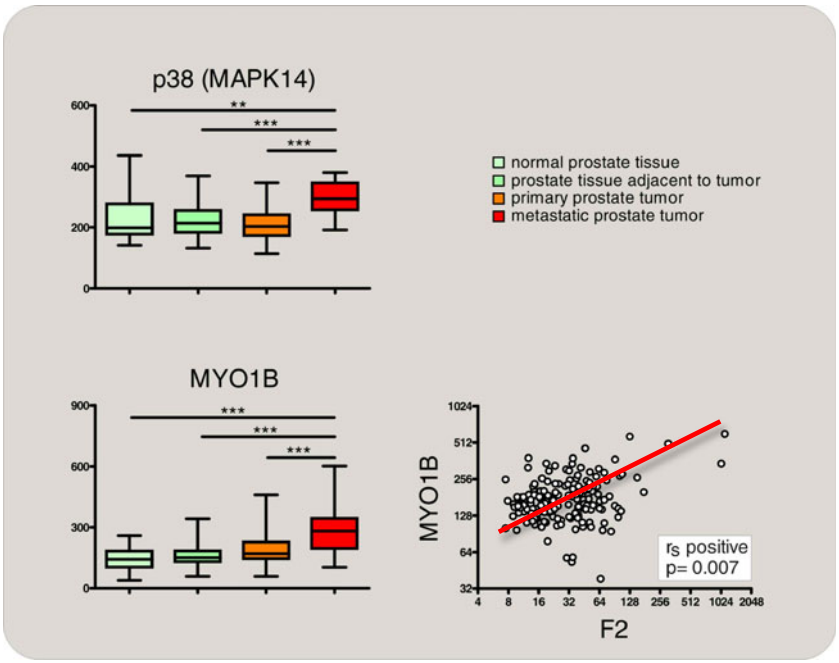

Fig. 6 Induction of thrombin (*F2*) gene expression correlates with a stage-dependent activation of p38 MAPK signaling in metastatic prostate cancer. Normalized mRNA expression of p38 MAPK (*upper diagram*) and of MYO1B (*lower left diagram*), which reflects activation of p38 MAPK signaling [165], obtained from gene expression profiling of 171 human samples [162] (median, *horizontal line;* 25th through 75th percentile, *box*; range, standard error of the mean (SEM); **p*<0.05; ***p*<0.01; ****p*<0.001). Correlation of p38 MAPK signaling activation (MYO1B gene expression) with F2 gene expression is shown in the lower right diagram (Spearman's rank correlation)

defects of placental angiogenesis [155]. Partly, this phenocopies the lethal vessel-malformation phenotype of mice lacking thrombin [53]. Interestingly, the functional importance of p38 MAPK for the regulation of thrombin gene expression is further corroborated in prostate cancer patients, where a stage-specific induction of p38 MAPK activity correlates with an up-modulation of thrombin gene expression in metastatic prostate cancer (Fig. 6).

Thrombin in blood coagulation, inflammation, cancer and beyond: back to Virchow?

Importantly, an estimated 40–50 % of all human cancers are linked to chronic inflammation [156], which can induce p38 MAPK. But also other environmentally triggered programs such as the DNA damage or oxidative stress response (with important roles during tumorigenesis) can directly activate

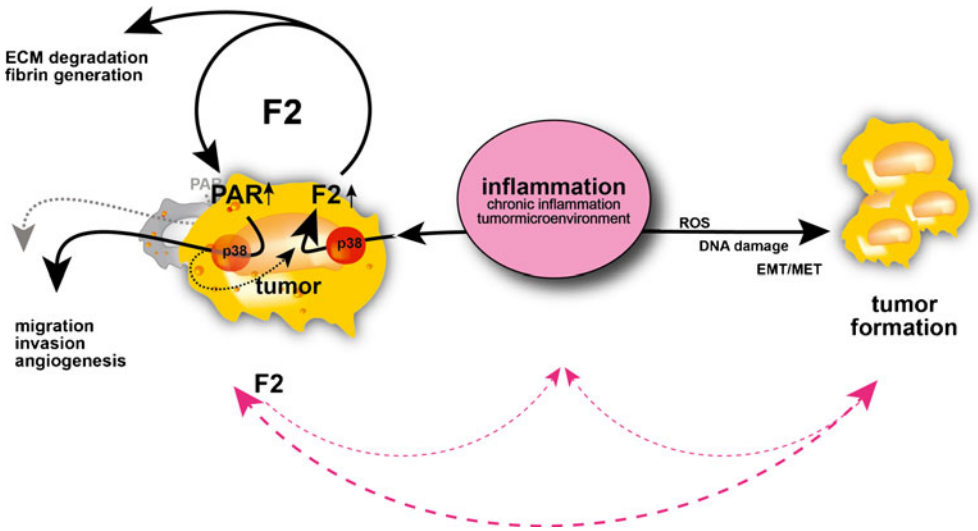

Fig. 7 Model for inflammation as a unifying trigger predisposing to deregulated blood coagulation (thrombin gene expression) and tumor formation. Inflammatory stimuli can induce both tumor formation (simplified) and thrombin (F2) gene expression. This in turn leads to a disequilibrium of pro- and anticoagulatory activities (and thereby promotes tumor-associated thrombus formation) and drives protumorigenic cellular programs (in an autocrine and/or paracrine manner; SD unpublished). Tumor formation will thus be supported by the tumor-promoting properties of thrombin; vice versa, tumor formation elicits detrimental inflammatory responses [159], which in turn further promote tumorigenesis and p38 MAPK (p38)-mediated induction of thrombin gene expression. (Extracellular matrix (*ECM*), Reactive oxygen species (ROS), epithelial–mesenchymal transformation, mesenchymal–epithelial transformation (EMT/MET))

p38 MAPK [154], and deregulated p38 MAPK signaling itself is associated with cancers in humans and mice [155, 157]. Therefore, protumorigenic signals such as inflammation, DNA damage, or reactive oxygen species might hypothetically represent the long-sought-after common risk factor between deregulated blood coagulation and the increased incidence of cancer [73] with detrimental bidirectional self-sustaining qualities [7, 82] (Fig. 7).

Remarkably, such an association is also corroborated by earlier studies, which demonstrated an inflammatory tumor microenvironment to be associated with the induction of thrombin expression [113]. This regulatory mechanism would provide a molecular basis by which protumorigenic environmental stimuli such as inflammation [158–160] can be directly linked to the hyperexpression of thrombin (and its detrimental consequences on tumor biology). This mechanism might also help to explain thrombin's role at the center of numerous inflammatory and noninflammatory (patho)physiological processes.

Future directions

Although we unquestionably have broadened our understanding of thrombin and its cellular functions within the past 20 years, we are just beginning to understand that thrombin plays a previously underappreciated role in (patho)physiology—ranging from autoimmunity, blood coagulation, cancer to wound healing, but possibly also regenerative medicine.

What remains puzzling is the contribution of thrombin to many of these processes on a systems' level in living organisms. This is mainly due to the fact that the complete lack of thrombin is lethal. In fact, most of the aforementioned roles of thrombin's contribution in diverse (patho)physiologies have been explored in cell culture studies and/or been deduced from animal studies where thrombin activity was reduced by pharmaceutical inhibitors. What continues to be challenging is the multifunctionality of thrombin: it can act systemically but also locally in different, though (patho)physiologically meaningful "sub-compartments." Thus, systemic determinations of thrombin activity do not necessarily reflect its local activity. In addition, there are numerous tissues in which thrombin is dynamically (re)expressed and modulated in various (patho)physiological conditions (see above). We currently do not understand to what extent (de)regulated thrombin expression and its activation represent cause or consequence of (patho)physiological processes. Therefore, studying the role of thrombin with spatial and temporal resolution could pave the way to much better dissect the roles of this multifunctional serine protease in various tissues and disease entities. This would also help elucidating the potential therapeutic dimension of strategies targeting thrombin gene expression and/or activation.

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