Haemostatic Proteins as Markers of Disease Progression and Prognosis in Breast Cancer

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Abstract

Background: Breast cancer is a leading cause of cancer death in women worldwide. One of the major causes of death from breast cancer is metastatic disease which results from the malignant cells invading and migrating through blood vessels to distant sites.

Text: Several studies have shown that metastasis is facilitated by haemostatic proteins. Breast cancer is characterized by haemostatic imbalance which is tilted more to a procoagulant state with resultant thrombotic complications. These elements that are involved in thrombosis also play key roles in different aspects of breast cancer growth including cancer proliferation and progression, cancer survival, angiogenesis and metastasis. Some of these elements include platelets, endothelial cells, coagulation factors and fibrinolytic proteins. There is a close relationship between cancer and many of the haemostatic elements. They are usually increased in metastatic breast cancer, and have found use as predictive and prognostic markers. Some have been validated in breast cancer. Due to their seemingly active roles in breast cancer progression, some of the haemostatic proteins are being developed as diagnostic tools in the management of breast cancer. They are equally being seen as potential targets for the development of novel therapies in breast cancer or repurposing drugs in current use for the same gain.

Conclusion: This review highlights the role haemostatic proteins play in breast cancer progression, and their diagnostic and therapeutic relevance.

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1. Introduction

Breast cancer (BC) is the most commonly diagnosed cancer in women around the world with more than 2.25 million new cases in 2020[1]. According to the GLOBOCAN 2020 data, the mortality rate of breast cancer was about 30%, and breast cancer also accounted for approximately 7% of all cancer deaths[2]. Majority of these deaths are as a result of metastatic disease[3]. Metastasis is a process involving the complex interplay between intrinsic tumor cell properties as well as interactions between cancer cells and multiple microenvironments which lead to the dissemination of cancerous cells to distant tissues with the development of discontiguous secondary mass which is independent of the original tumor. It is reported to account for two-third of deaths in cancer patients[4]; and more than 50% of patients who present with breast cancer exhibit osseous metastasis according to a population-based study[5]. The process of metastasis is not a spontaneous event, but rather a sequence of events which is initiated by the genetic instability of the tumor cell as a result of accumulated mutations through which the cell acquire invasive properties up to colonization of new sites[6]. This is not a one-off thing as several factors contribute to the sequences of events. While mechanistically complex, there is a search to identify genes and protein that are involved in the initiation and progression of metastasis especially in breast cancer patients. These genes and proteins include the tumor protein p53 (*TP53*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*), phosphatase and tensin homolog (*PTEN*), retinoblastoma (*RB1*), Hypoxia-inducible factors (HIF), Matrix metalloproteinases (MMPs) etc[7][8][9][10]. Besides the mentioned factors, the host hemostatic system is increasingly recognized as an important regulator of breast cancer progression. The haemostatic system which consists of platelets, coagulation and fibrinolysis is known to affect different processes in the progression of breast cancer. In addition, some aspect of cancer development and progression such as angiogenesis, immune evasion, anti-apoptosis, and tumor invasion have strong association with the haemostatic system[11][12][13][14]. This invariably lead to venous thromboembolism (VTE), a condition that lead to a three to fourfold increase in breast cancer patients compared to age-matched women without cancer[15]. About 50% of cancer patients, and up to 90% of patients with metastatic disease exhibit coagulation abnormalities which is reflected in their laboratory tests. Histopathological analyses show the presence of fibrin and platelet aggregates in and around various types of tumors, implying the activation of the coagulation cascade. Cancer cells have been shown to express some hemostatic factors as well as endogenously synthesize others[16][17].

Circulating tumour cells (CTCs) are cells from a primary tumour that have entered the vasculature or lymphatics, and are the principal mechanism for development of metastases. Kirwan et al showed that the presence of CTCs was significantly associated with increased levels of fibrinogen, D-dimer, thrombin–antithrombin III (TAT) and reduced overall survival in metastatic breast cancer patients[18]. Additionally, the CTC-platelets interaction have been shown to transfer the major histocompatibility complex to CTCs[19], which cause CTCs to mimic host cells and confound the immune cells, act as a physical barrier against immune cells[20], stimulate and accelerate epithelial to mesenchymal transition in CTCs through the secretion of some growth factors[21], and increase CTCs adherence to the cell wall through the activation of the

platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31)[22]. In another study, high platelet counts were also shown to be associated with supraclavicular lymph node metastasis and poor prognosis in breast cancer patients[23]. These studies show the association between the hemostatic system and breast cancer progression.

I delved into the complex relationship between haemostatic factors and breast cancer and delineate the important role played by platelets, coagulation, and fibrinolytic proteins in tumour metastasis in breast cancer patients. I also highlighted the potentials of targeting haemostatic factors for the management of breast cancer and prevention of disease progression.

2. Overview of haemostasis

The haemostatic system is a complex and delicate control in the human physiology that under normal circumstances maintains blood in a fluid state within the body circulation, but can initiate a cascade of events called coagulation when there is tissue injury in order to minimize blood loss. If this fails, a dysfunctional haemostasis can lead to prolonged bleeding; on the other hand, excessive coagulation which is also dysfunctional can lead to thrombosis. The word haemostasis is said to have originated from two Greek words “heme” which means blood, and stasis which means “to stand still”. The process of haemostasis is well-regulated and it involves many cellular and acellular components. The cellular components include: the blood cells and cells of the vascular system. These cells include red blood cells[24], granulocytes[25], lymphocytes[26] and platelets[27][28]. The cells of the vascular system include the endothelial cells, smooth muscle, and connective tissue[29], whereas the acellular components include coagulation factors, fibrinolysis system, kinin system, complement system, and serine protease inhibitors. All these components work in a stochastic manner to arrest bleeding, and the erstwhile formation of a blood clot. The process of haemostasis can thus be divided into four steps, the first being three being involved primarily in clot formation. These steps are:

A. Vasoconstriction of blood vessel
B. Temporary formation of a platelet plug
C. Activation of the coagulation cascade
D. Formation of a fibrin plug/ fibrinolysis

Upon vascular damage from any insult, vasoconstriction of the affected blood vessel happens almost immediately through the stimulation of the sympathetic nervous system of the smooth muscle cells after the release of vasoactive agents such as endothelin-1, bradykinins, vasopressin, and histamines by the endothelial cells. This action causes a decrease in blood flow through the affected vessel by reduction of the vessel diameter. This action turns a constitutively anticoagulant endothelium to a procoagulant one in order to reduce blood loss. Upon the immediate vasoconstriction of the blood vessel following injury to the endothelium, vasoactive agents, such as bradykinin and histamine release cause the release of von Willebrand factor (VWF) from the injured site. von Willebrand factor is a multimeric protein that is synthesized by endothelial cells and stored in specialized granules within the endothelial cells called Weibel- Palade bodies. VWF subsequently binds through its A3- domain to subendothelial structures forming the bridge between platelets and

endothelial cells. This then lead to platelet adhesion either to the VWF or the exposed subendothelial structures, namely collagen and laminin. During this process, platelets undergo changes in shape with the release of its granules. This then lead to further platelet recruitment and aggregation leading to the formation of a temporary platelet plug. At the same time, activated platelets adhere to subendothelial structures and VWF through its glycoprotein receptors. Adhesion to subendothelial collagen is performed by the two main platelet glycoproteins (GP) which are GPIa/IIa (integrin α2β1, CD49b/CD29) and GPVI. Platelets also interact with VWF through its GPIb/V/IX (CD42a-c) which binds to the A1-domain of VWF. There is a further platelet-platelet interaction which is mediated by integrin αIIbβ3 (GPIIb/IIIa). GPIIb/IIIa is the most abundant GP on platelets and it plays an important role in platelet-platelet interaction otherwise called aggregation which is the interaction between fibrinogen and GPIIb/IIIa on opposite platelets to enhance platelet aggregation for the formation of the platelet plug.

Simultaneous to platelet activation, the coagulation cascade is initiated through two pathways namely: (i) the extrinsic (tissue factor) pathway, and (ii) the intrinsic (contact) pathway. The extrinsic pathway involves the conversion of FVII to FVIIa. This is achieved through the activated endothelial cells which act by the release of tissue factor (TF;factor III) in response to injury to the vasculature. To prevent the initiation of coagulation, the intact endothelial cells express tissue factor pathway inhibitor (TFPI), a serine protease that dampens activation of clotting through the extrinsic pathway by serving as a factor Xa (fXa)–dependent inhibitor of TF–factor VIIa. However, when there is a vascular injury, TF which is a transmembrane glycoprotein found in the adventitial layer of the blood vessel is exposed to coagulation factors in the blood, and directly interacts with factor VII. The TF-VIIa complex interacts with the zymogen factor X and IX converting them to factor Xa and IXa respectively. TF acts as a regulatory subunit while factor VII catalytically converts factor X to Xa or factor IX to IXa. Factor IXa further enhances the activation of factor X which subsequently converts the zymogen prothrombin (factor II) into to thrombin (factor IIa) in the presence of its cofactor FVa and phosphatidylserine (the “prothrombinase complex”).

On the other hand, coagulation also occur when blood is exposed to negatively charged surfaces, with the activation of factor XII by high molecular weight kininogen (HMWK) and prekallikrein[25]. Auto-activation of factor XII also occurs when it comes in contact with such substances as polyphosphates, nucleic acids, heparin, collagen, misfolded proteins, extracellular traps, and anionic bacterial surfaces[26][27][28]. Also, prekallikrein can be directly activated by factor XII to kallikrein, which in turn can convert more factors XII to XIIa giving a positive feedback. This is the “extrinsic tenase” complex. Factor XIIa activates factor XI to its activated form factor XIa. It should be noted that in all these that HMWK is involved, and it is also converted to kinins which can cause vasodilation, erythema and pain. Factor XIa subsequently activates factor IX to IXa with calcium ions as a cofactor. Factor XIa together with factor VIIIa, phospholipids and calcium ions – “ the intrinsic tenase complex” converts factor X to Xa.

The generated thrombin as a result of the action of the “prothrombinase complex” cleaves fibrinogen to become fibrin. Thereafter, fibrin polymerization occurs with the buildup of the fibrin monomers to a polymeric fibrin which forms a meshwork with the aggregating platelets to form a clot. To strengthen this fibrin meshwork, factor XIII – also known as fibrin stabilizing factor, a transglutaminase enzyme which is activated by thrombin (Factor XIII to XIIIa) forms gamma-glutamyl-lysyl amide crosslinking of fibrin thereby stabilizing the fibrin clot to withstand shear stress. Simultaneously,

thrombin is involved in the activation of thrombin-activatable fibrinolysis inhibitor (TAFI) which protects the newly formed fibrin clot from being lysed by fibrinolytic proteins.

Thrombin is central to many actions in the process of coagulation; hence, excess thrombin can induce a pathological thrombotic state[29]. Thus, a number of anticoagulation mechanisms regulate thrombin generation. These include antithrombin, protein C inhibitor, heparin cofactor II, protease nexin 1, cartilage oligomeric matrix protein and TFPI[30][31][32].

The final process of haemostasis which is fibrinolysis is initiated by the release of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) that convert plasminogen into plasmin, which initiate fibrin lysis leading to formation of fibrin degradation products. However, some fibrinolytic inhibitors can impede the activity of plasminogen activators including plasminogen activator inhibitors (PAI-1), alpha 2-antiplasmin, and TAFI (which causes increased resistance to fibrinolysis by tPA)[33]. (Figure 1).

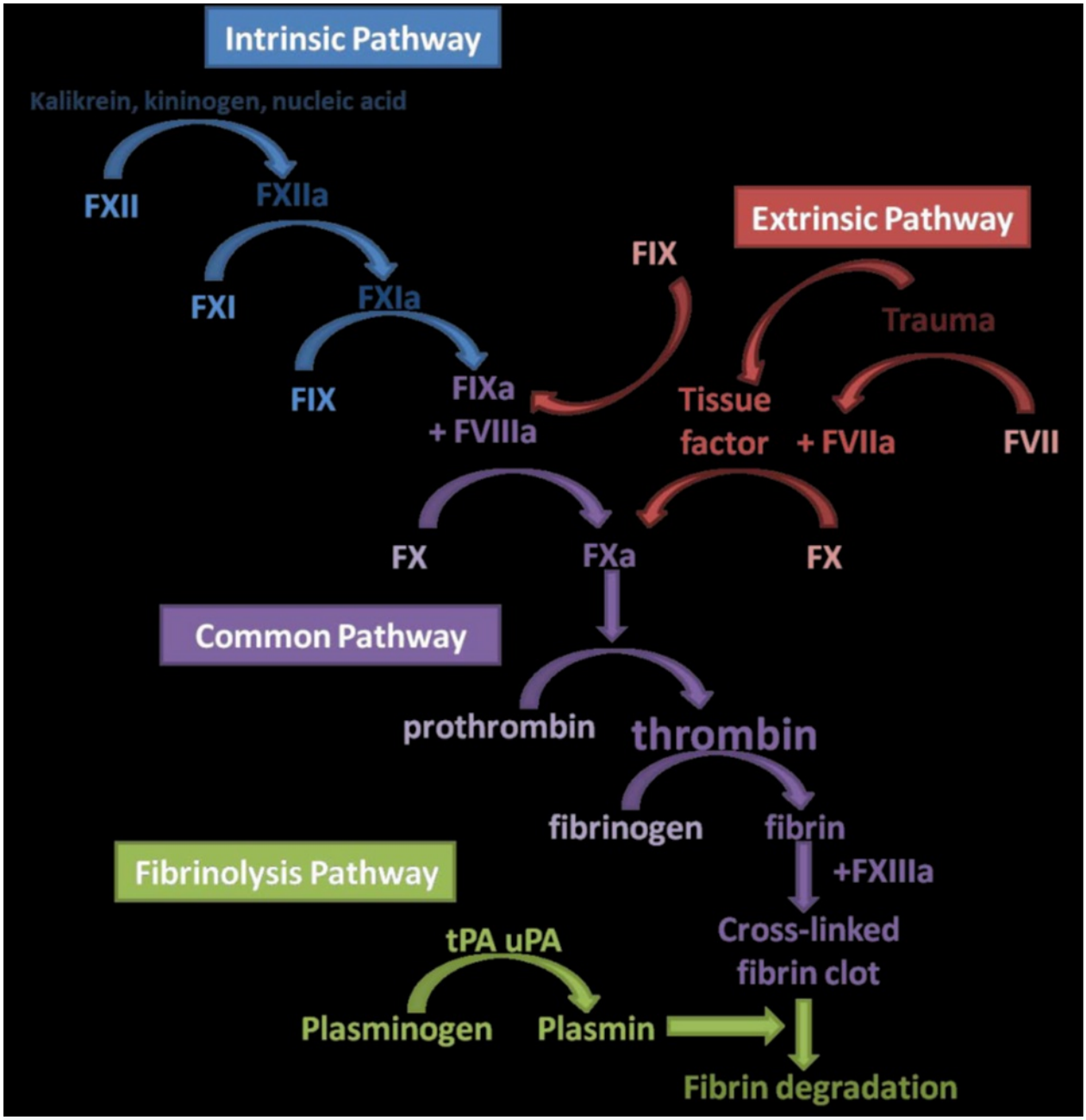

Figure 1. An overview of the coagulation cascade showing the various haemostatic pathways involved in haemostasis.

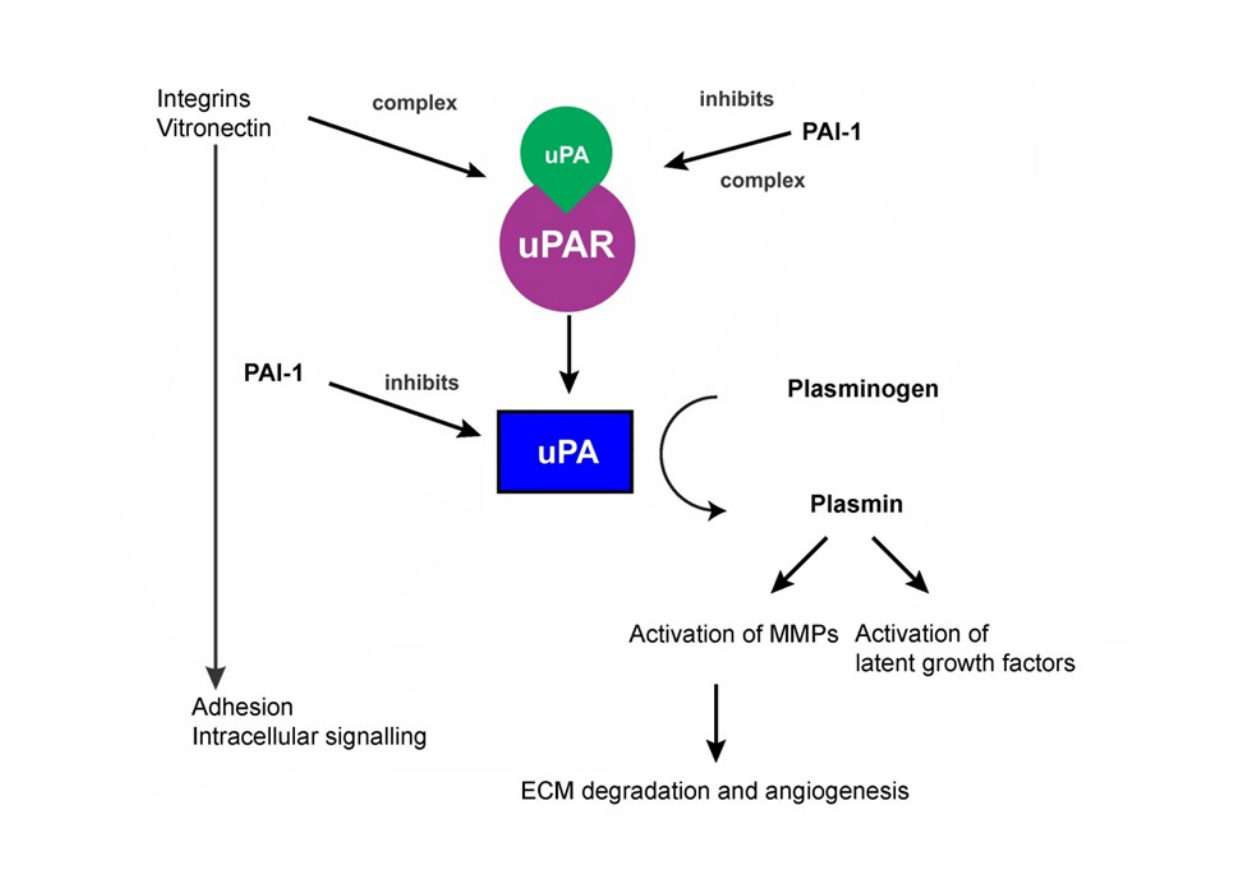

Figure 2. The uPA, uPAR, and PAI-1 interaction in the fibrinolytic process that promotes breast cancer progression.

3. Breast cancer and haemostasis

Breast cancer like every other cancer is an acquired hypercoagulable state that is characterized by abnormal laboratory results, and thus, enhancement of the coagulation cascade as well as platelets. Apart from activation of the coagulation cascade, there is also the failure of physiological anticoagulant agents and also loss of integrity of the vascular endothelium due to the tumour cells which enhances a hypercoagulable state. Evidences for this abound. Histopathological analyses show the presence platelet aggregates and fibrin deposits in and around various tumour types, an indication of local blood activation; with between 60 to 100% of patients with cancer reported to have haemostatic alterations without any accompanying thrombotic events[34]. This interaction between cancer cells and the vascular endothelium along with some haemostatic proteins is primarily responsible for the pro-thrombotic and pro-inflammatory events in cancer. These haemostatic alterations also form a crosstalk between thrombus formation and inflammatory response. For example, fibrin is known to act as a scaffold for platelets and leukocytes to bind, release inflammatory signals and play a role in wound healing[35]. Fibrin(ogen) has also been reported to induce production of proinflammatory signaling molecules by leukocytes[36][37][38]. Thus elevated coagulation factors and inflammatory markers are a risk factor for cancer-associated thrombosis[39].

This hypercoagulability state is known to influence the biology of cancer. In breast cancer, the haemostatic activation have

been shown to be more than just an effect of cancer progression, but also a major regulator of malignant transformation, tumour angiogenesis, and metastasis[40]. Fibrin is also known to provide a scaffold for tumor cell anchorage and invasion, thus, protecting the cancer cell from immune recognition. Thrombin has also been shown to promote invasive growth and metastasis of cancer cells[41][42]. D-dimer, the end-product of fibrinogen hydrolysis is also associated with tumour progression in different cancers including breast cancer[43][44]. Thus, the hemostatic alterations associated in breast cancer progression like other cancers are expressed in different forms including prolonged and shortened prothrombin time (PT), activated partial thromboplastin time (APTT), increased and decreased levels of thrombin, and other coagulation proteins and thrombocytosis. These alterations have been shown to be closely associated with tumour burden as well as tissue metastasis. This chapter will therefore examine different haemostatic system, discuss their functions, and the important role each play in the progression of breast cancer.

4. Platelets and breast cancer

Although they are not proteins, platelets are anucleate discoid shaped cells measuring between 1.5-3 μm in size. As earlier mentioned, they are known to play an important role in vascular homeostasis; furthermore, they are also involved in inflammation, sepsis, wound healing and immunity. Their primary physiological role is to prevent or stop bleeding through the formation of a clot after they have been activated. However, in pathological conditions like cancer, this role can be dysregulated. Evidences have shown that platelets play a role in breast cancer progression[45][46]. One of such evidence is thrombocytosis, which has been shown to be associated with solid tumours for more than a century. Platelet count has also been shown to be closely associated with tumour size and stage in breast cancer[47]. Gasic et al used an animal model to show a 50% reduction in tumor metastasis after experimental thrombocytopenia induced with neuraminidase and anti-platelet serum which was reversed with the introduction of platelet-rich plasma[48]. Thrombocytosis is thus recognized as a poor prognostic factor in breast cancer patients. Platelets are involved in breast cancer progression and metastasis through different mechanisms including the promotion of tumour angiogenesis and the facilitation of tumour extravasation through the epithelial-mesenchymal transition; also with increased survival of tumour cells in circulation by aiding them to evade immune recognition and phagocytosis. Under physiological conditions, platelet activation from a stimuli leads to shape change and degranulation. The platelets' α-granules and dense granules then release different substances that affect the body's homeostasis. Tumour cells take advantage of this through a process called tumour cell-induced platelet aggregation (TCIPA). This can happen by tumour cell induced thrombin generation or through the release of several mediators from the activated platelets that induce platelet aggregation and other vascular changes that promote the growth, survival, motility, and the extravasation of circulating cancer cells. These mediators include ADP and thromboxane A2 which have been shown to induce platelet aggregation in vitro[49][50].

Breast cancer cells can also stimulate platelet aggregation via generation of thrombin. The thrombin generated activates platelets through the protease–activated receptors (PAR) on platelets; PAR 1-4, PAR–1 being the most potent receptor for thrombin. PAR-1 is also observed to be elevated in breast cancer with associated cancer progression and poor prognosis[51]. Thrombin–activated platelets express substances that facilitate contact with tumour cells and in turn

enhance TCIPA. TCIPA is also enhanced via the activation of matrix metalloproteinase-2 (MMP-2) at the cell surface which is mediated by membrane type-1 matrix metalloproteinase (MT1-MMP). This interaction usually involves the integrin αvβ3, and absence of the integrin can translate to reduced platelet aggregation and platelet-cancer cell interaction[52]. This activated platelet can release more substances that play a role in breast cancer metastasis.

One of such roles is angiogenesis. Angiogenesis is an important event in the initiation and metastasis of cancer cells. A fall in blood supply means tumours cells' growth would be retarded, and eventually die. The process of tumour angiogenesis in breast cancer is a complex interplay of tumour cells, and the tumour microenvironment. It involves the secretion of some growth factors, and angiogenic factors all of which are present in the alpha granules of platelets. Some of these growth factors include: Platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF) etc. Elevated levels of VEGF have been shown to activate the VEGF pathway thereby causing the formation of new blood vessels and breast cancer cells proliferation. The process of angiogenic factors release from platelets is usually inflammation driven, and in breast cancer may involve inflammatory markers synthesized from the cancer cells such interleukin-6. This process of VEGF release from platelets have been observed in breast cancer patients; in addition, platelets from breast cancer patients release significantly more VEGF than those from healthy controls and also correlate with serum VEGF[53][54]. The platelet pool of VEGF is reported to consist of more than 80% of total circulating VEGF in healthy subjects as well as in breast cancer patients[55]. As tumour cells circulate in the bloodstream, they are known to be shielded by platelets, which at some point in the endothelium release their VEGF to induce endothelial wall permeability, along with angiogenesis, thus, aiding cancer metastasis. Experimental works in breast cancer shows that platelets angiogenic activities are mediated by VEGF–integrin cooperative signaling via PI3K/PKC pathway[56]. Apart from the pro-angiogenic proteins like VEGF, platelets also store anti-angiogenic proteins (Table 1). These include endostatin, platelet factor-4, thrombospondin-1, α2-macroglobulin, plasminogen activator inhibitor-1, and angiostatin. The pro-angiogenic and the anti-angiogenic proteins have been shown to be stored in different compartments of the α-granules, and are selectively released upon different stimuli[57][58]. Both angiostatin and endostatin have been shown to exhibit anti-tumoral efficacy. In addition, platelets have been shown to regulate vascular integrity and prevent tumour bleeding especially in murine models of breast cancer.

Table 1. Some of the granule contents of platelets that are involved in angiogenesis.

Granule type (Platelets)	Content	Role in angiogenesis
α-granules (growth factors)	1. Platelet-derived growth factor (PDGF)	Promote
	2. Vascular endothelial growth factor (VEGF)	Promote
	3. Basic fibroblast growth factor (bFGF)	Promote
	4. Epidermal growth factor (EGF)	Promote
	5. Interleukin 1-Beta (IL-1 β)	Promote
	6. Stromal cell-derived factor-1 α (SDF-1 α)	Promote
	7. Angiopoietins	Suppress
	8. Platelet factor 4	Suppress
	9. Endostatins	Suppress
	10. Thrombospondin 1	Suppress
	11. Sphingosine-1-phosphate	Suppress
	12. TIMPs (TIMP 1,2 and 4))	Suppress
	13. MMPs (1,2,3,9 and 14)	Suppress
	14. HGF (Hepatocyte growth factor)	Suppress
	15. Transforming growth factor-β (TGF-β)	Suppress
α-granules (haemostaticfactors)	1. Factor V	Promote
	2. von Willebrand factor (VWF)	Promote
	3. Fibrinogen	Promote
	4. Factor XIII	Promote
	5. Plasminogen	Promote
	6. Tissue factor pathway inhibitor (TAFI)	Suppress
	7. Antithrombin	Suppress
	8. Protein S	Suppress
	9. Plasminogen activator inhibitor-1(PAI-1)	Promote
Dense granules	1. Adenosine triphosphate (ATP)	Promote
	2. Adenosine diphosphate (ADP)	Promote
	3. Guanosine 5′-triphosphate (GTP)	Promote
	4. Guanosine diphosphate (GDP)	Promote

Table 2. Role of Hemostatic Proteins in Breast Cancer Progression

Hemostatic protein	Role in breast cancer
Tissue factor	Cell migration, anti-apoptosis, angiogenesis
Thrombin	Cell extravasation, angiogenesis
Fibrinogen	Cell extravasation, lymph node metastasis
D-dimer	Cell extravasation, lymph node metastasis
UPAR	Cell adhesion, Cell migration, angiogenesis
uPA	Cell adhesion, Cell migration, angiogenesis
PAI-1	Cell migration, cancer relapse
vWF	Cell metastasis, angiogenesis, cancer relapse
PAF	Cell metastasis, angiogenesis

VWF – Von Willebrand factor; PAF – Platelet aggregating factor; PAI-1 - Plasminogen activator inhibitor-1; UPAR - urokinase-type plasminogen activator receptor; UPA - urokinase-type plasminogen activator.

While in circulation, circulating tumour cells (CTCs) can easily be targeted and destroyed by immune cells, however, the platelet-tumour cell interaction helps protect some CTCs from the cell death induced by natural killer (NK) cells. Platelets have been shown to act in a number of ways to prevent CTCs cell death which include: 1) interaction between the breast cancer cells and platelets has been shown to activate the TGF-β signaling pathway which promotes metastasis and also cause immunosuppression by downregulation of DNAM-1 (CD226) and TACTILE (CD96) expression in NK cells. 2) Breast cancer cells can also ‘pick up’ MHC (major histocompatibility class 1) antigens of platelets which help them evade NK cell recognition as foreign cells, limiting NK cell cytotoxicity. 3) Platelets have been shown to induce tumour cells to produce ligands such as NKG2D which prevents NK cell recognition and subsequent NK cell cytotoxicity. 4) Another mechanism is by platelets binding to mucins on the surface of cancer cells through the GPIb-IX-V and GPIIb-IIIa adhesion molecules that are mediated by surface integrins αVβ3 or P-selectin on the cancer cells which can create a physical barrier that prevents NK cell from destroying it.

Platelets are involved in clot formation through their interaction with the endothelium. This interaction can also facilitate tumour cell extravasation to other sites. GPIIb/IIIa and P-selectin play a major role in the platelets-tumour cells adhesion. Zhao et al in their work showed that GPIIb/IIIa was important for hematogenous metastasis of human breast carcinoma MDA-MB-231 cells[59]. Research data has also shown that the platelet-tumour cell interaction through a P-selectin mediated interaction plays a role in tumour extravasation in a similar way to leukocyte diapedesis with endothelial cells[60]. Others like GPIb/IX have also been implicated in metastasis in a VWF-mediated adhesion[61][62].

With the established evidence of platelets involvement in breast cancer and other cancers’ metastasis, there has been a drive into the use of antiplatelet therapies in the management of cancer progression. However, this has been met with mixed results. In a recent phase III trial (NCT02927249) of aspirin use in breast cancer survivors, there was no benefit and

thus, aspirin was not recommended for breast cancer prevention[62]. In a recent study of women with inflammatory breast cancer, it was shown that the use of aspirin was associated with both OS (overall survival) and DFS (disease-free survival) benefits[63]. Kononczuk et al demonstrated that abciximab and eptifibatide (GPIIb-IIIa inhibitors) can induce MCF-7 breast cancer cell apoptosis[64]. These effects including the prevention of haematogenous metastasis have been demonstrated in other cancer cell lines as well. Platelets, due to their close interaction with tumour cells especially the tumour microenvironment have also been as a potential drug delivery system. Yap et al in their work conjugated monomethyl auristatin E (MMAE), a microtubule inhibitor to a single chain antibody (scFv) targeting platelet GPIIb-IIIa. The antibody-drug conjugate successfully targeted MDA-MB-231 tumour in a murine model leading to a significant decrease in tumour growth compared to untreated mice and mice treated with MMAE conjugated to a non-binding antibody control. This, and similar preclinical works has shown the role platelets play in breast cancer progression, and can be exploited for therapeutic benefits[65][66][67].

5. The tissue factor pathway and breast cancer

Tissue factor (TF) is a 47-kDa integral membrane glycoprotein that initiates the extrinsic pathway by forming a complex with factor VIIa. TF is known to act as the regulatory subunit of the TF:FVIIa complex, while the factor VIIa serine protease domain which is bound to TF is involved in the activation of downstream coagulation factors most especially factor X. aside its haemostatic function, the TF:FVIIa complex can also activate inflammatory pathways by binding to protease-activated receptors 1 and 2 (PAR-1 and PAR-2) which play a role in angiogenesis, and tumour invasion. The TF gene which is located on chromosome 1, p21–p22 and is made up of a 219-amino-acid extracellular domain, a 23-amino-acid transmembrane segment and a 21-amino-acid cytoplasmic tail is regulated by several transcription factors that are associated with hypoxia and inflammation including nuclear factor-κB (NF-κB) and activator protein (AP-1). TF is constitutively expressed in subendothelial cells, and it only interacts with blood when there is loss of vascular integrity. While the TF/fVIIa/fXa complex is a recognized player in vascular haemostasis, several studies have shown it plays a major role in cancer progression especially breast cancer[68][69][70][71][72]. It is also associated with poor survival across many cancer types[68][73]. However, Stampfli et al did not find any association between TF and survival in breast cancer patients[74].

As earlier mentioned, TF can activate PARs. The PARs are a 4 member subfamily of G protein-coupled receptors (GPCRs) that are activated by proteolytic cleavage of the extracellular amino terminus. They are involved in haemostasis, angiogenesis, inflammation, neural tube closure, cell growth etc. Both TF/FVIIa complex and TF/FVIIa/FXa complex are also known to mediate this TF signaling and others. Although the PARs are primarily thrombin receptors, TF/FVIIa/FXa complex can equally cleave and activate PAR1; it is equally cleaved by MMP-1, plasmin and activated protein C. PAR2 is not directly activated by thrombin unlike other PARs[75]. TF/FVIIa and the TF/FVIIa/FXa complex can directly cleave it. The TF/FVIIa complex can trigger a transient increase in calcium which can lead to the activation of the Mitogen-activated protein kinases (MAPKs) pathway including p44/42, p38, and C-Jun N-terminal kinase (JNK) that play a major role in cell cycle control, and the Src-like kinases, PI3 kinase, the JAK/STAT pathway, and the Rho GTPases Rac1 and Cdc42 that

are involved in cell survival and cytoskeletal rearrangements[69][76]. Apart from the MAPK, PI3 kinase and JAK/STAT pathway, TF/FVIIa complex also targets the RTK, IGF-1 and integrin signaling. TF association with integrin has been shown to play a role in breast cancer metastasis and cell survival[77][78]. Breast cancer cells have also been shown to inhibit apoptosis via phosphorylation of the p44/42 MAPK and Akt/ protein kinase B signaling through the TF-FVIIa-FXa complex rather than thrombin, activating the mammalian target of rapamycin (mTOR) pathway which results in cell migration, an important step in metastasis; inhibition of this mTOR pathway reduced cell migration significantly[79][80]. In a similar experiment, simvastatin was also able to inhibit the phosphorylation of Akt/ protein kinase B in a breast cancer cell line by abrogating the TF/FVIIa and TF/FVIIa/FXa complex signaling preventing cell proliferation[81]. Through these pathways, TF is involved in breast cancer metastasis, cell proliferation and survival. Tumour-expressed FVII is also reported to enhance tumour growth and metastasis in breast cancer[82].

TF signaling induce angiogenesis either through a clotting-dependent means or a clotting-independent way. The clotting-independent way involves PAR 2 signaling. This is usually achieved through the cytoplasmic domain of TF. The clotting-dependent means involves the TF-induced thrombin formation and deposition of cross-linked fibrin which creates a pro-angiogenic template that aids the infiltration of blood vessels. The overexpression of TF has also been shown to enhance cancer cell growth through the increased transcription of VEGF, and reduced transcription of thrombospondins[83]. VEGF is reported to mediate TF expression via early growth response protein 1(EGR1)[84].

TF has been discovered to exist in three isoforms which include a full length TF earlier described (a 219-amino-acid extracellular domain, a 23-amino-acid transmembrane segment and a 21-amino-acid cytoplasmic tail); an alternatively spliced tissue factor (asTF) isoform which is generated via the omission of exon 5 during the processing of TF's primary transcript which causes a shift in the reading frame. The asTF protein has a unique C-terminus that lacks a transmembrane domain which ultimately makes it soluble[85]; and the alternative exon1A-tissue factor (TF-A) which is produced by an alternative splicing mechanism involving the first intron. While not so pertinent to haemostasis, the asTF has been shown to be produced by different cancers, including breast cancer cell lines[86]. The asTF isoform has been shown to promote breast cancer progression and metastasis, tumour angiogenesis most often in a beta integrin-dependent manner[87][88].

Due to its high expression in different cancer cells and the multiple roles it plays in such cancers, TF have been muted as a target for cancer therapy. These include the use of different kinds of antibody technologies which has yielded results in different settings[89][90]. The recent approval of the first TF-targeted antibody-drug conjugate, tisotumab vedotin ((HuMax®-TF-ADC)) for cervical cancer has given for the use of antibodies against TF in breast cancer. The NCT03485209 a phase 2 clinical trial is currently recruiting patients with solid tumours, although breast cancer is not included. Since TF acts mainly through PARs, blocking PAR signaling has also been seen as a therapeutic strategy. Atopaxar and vorapaxar, two PAR-1 inhibitors have been shown to inhibit cancer progression in preclinical models[91]. The monoclonal antibody Mab5G9 that blocks TF-VIIa mediated activation of PAR2, and also disrupts the interaction of TF with integrins have also been shown to have antineoplastic activity in breast cancer pre-clinical models[92]. Whether they can prove efficacious in breast cancer patients is yet to be determined. Factor VII and X are known vitamin K-

dependent serine proteases, therefore targeting them with the use of anticoagulants is a therapeutic strategy. Vitamin K antagonists, like warfarin, which has been shown to have antitumour potentials through antiangionesis, antiadhesion and decreased cell mobility[93][94]. A factor Xa inhibitor, rivaroxaban, was assessed for its antineoplastic properties in a phase 2 clinical trial with breast cancer patients (EudraCT 2014-004909-33), however no results are available[95]. But, rivaroxaban have been shown to promotes antitumor immunity by enhancing infiltration of dendritic cells and cytotoxic T cells at the tumor site, blocking factor Xa PAR 2 signaling[96]. Amblyomin-X a Kunitz-type FXa inhibitor discovered through the transcriptome analysis of the salivary gland from Amblyomma sculptum tick have also been shown to have potential antineoplastic activity[97]. Using TFPI pathway, a natural inhibitor of TF is also believed to an anticancer strategy in breast cancer progression[98]. Overall, the TF signaling which is a marker of breast cancer progression can be targeted in developing treatments for breast cancer.

6. Thrombin and breast cancer

Thrombin is a Na+-activated allosteric serine protease of the chymotrypsin family which is derived from its inactive zymogen form prothrombin, a 70-kd glycoprotein that is synthesized in the liver and secreted into the blood. Once generated from prothrombin (proteolytic cleavage by factor Xa), it plays two important roles. It acts as a procoagulant through the activation of fibrinogen to fibrin, activation of factors V, VIII, XIII and XI to Va, VIIIa, XIa and XIIIa respectively. It also inhibits the process of fibrinolysis via the activation of TAFI as well as activates platelets through PAR-1. On the other hand, it acts as an anti-coagulant when it binds to thrombomodulin, a receptor on the membrane of endothelial cells which suppresses its ability to cleave fibrinogen and PAR-1, but enhances its specificity towards the zymogen protein C, converting it to an activated protein C (APC). The generation of thrombin is a key step in blood coagulation. Thrombin acts by the cleavage of PAR-1, PAR-3 and PAR-4 particularly at a specific site within the extracellular N-terminus thereby exposing a tethered ligand, which subsequently folds back to activate the receptor. The cleavage of PAR-1 and PAR-4 leads to platelet activation and aggregation. PAR3 is not present on human platelets, but is widely and abundantly expressed in other cell types.

Several studies have shown that thrombin is involved in tumour growth, metastasis, and angiogenesis in different cancer models[99]. It is also reported to increase breast cancer invasiveness in some models. In the prospective HYPERCAN study, thrombin was shown to predict early recurrence in breast cancer, implying its role in tumour growth[100][101]. PAR-1, the primary receptor of thrombin is involved in cancer cell invasion and metastasis in multiple cell lines including breast cancer. PAR-1 is equally overexpressed in breast cancer. Wang et al in their study showed that TWIST- mediated induction of PAR-1 can promote epithelial-mesenchymal transition (EMT), tumourigenicity and metastasis by controlling the Hippo pathway[102]. This has made PAR-1 to be seen as a potential therapeutic target in cancer. In pre-clinical study of breast cancer cell lines, inhibition of PAR-1 resulted in a significant reduction in tumour growth and metastatic lesions[103][104][105]. Thrombin has also been targeted. The direct oral thrombin inhibitor, dabigatran have been reported previously to reduce breast cancer progression[106]. Dabigatran was equally reported to act synergistically with cyclophosphamide to inhibit breast cancer cell growth and metastasis in vivo[107]. However, in a recent study, Smeda et al

reported that dabigatran promoted pulmonary metastasis in a murine model of breast cancer[108]. Buijs et al also noted it did not inhibit metastasis in mouse models[109]. In a systematic review by Najidh et al, they concluded that direct oral anticoagulants (DOACs) do not affect tumour progression or metastasis in xenograft models[110]. Some of the DOACs have however been observed to have antineoplastic and anti-inflammatory properties[111]. The heparins, polysaccharides that belong to the glycosaminoglycan (GAG) family with the ability to bind antithrombin III (ATIII) and increasing its inhibitory effect on thrombin and factor X is known to induce a two- to four-fold increase in the level of circulating TFPI which has both anti-angiogenic and anti-metastatic properties. Heparin and its derivatives have been shown to inhibit lung metastasis in breast cancer models. The low molecular weight heparin, tinzaparin, has been shown to exhibit anti-metastatic properties in breast cancer cell lines[112]. Hirudin, a direct thrombin inhibitor has also been shown to anti-metastatic properties[113].

7. Fibrinogen, D-dimer and breast cancer

Fibrinogen molecules are about 45nm in length and are made up of two outer D domains, each connected by a coiled-coil segment to its central E domain. It is a large complex glycoprotein which consist of three pairs of polypeptide chains, designated as Aα, Bβ, and γ, with molecular masses of 66.2, 54.5, and 48.4 kDa, respectively, for a total mass of about 340 kDa, including the posttranslational addition of asparagine-linked carbohydrate to the Bβ– and γ-chains. Synthesized in the liver, fibrinogen play a host of roles in the human physiology. This include formation of blood clot, fibrinolysis, inflammation, wound healing, cellular and matrix interactions, and also neoplasms. The D-dimer is a unique marker of fibrin degradation. It is a product of polymerized fibrin (cleaved fibrinogen catalyzed by factor XIII) degraded by plasmin during fibrinolysis. Unlike other fibrin degradation products (FDPs) that show only plasmin activation, D-dimer show that there is activation of thrombin and plasmin, and also specific for coagulation and fibrinolysis. This unique quality have made D-dimer a variable tool in different clinical scenarios most especially in venous thromboembolism (VTE). Fibrinogen and D-dimer are perhaps the most studied haemostatic markers in cancers, and this may be due to their ready availability and low cost. Several studies carried out in cancer patient have shown that both fibrinogen and D-dimer markers of cancer progression and prognostic indicators. Plasma D-dimer has been shown to be significantly associated with clinical stage of breast cancer[114][115][116][117][118][119]. It also correlated significantly with histological stage[120][121]. Kirwan et al, also established that there is a significant association between elevated D-dimer levels and circulating tumour cells (CTCs) in metastatic breast cancer[122]. This has also been validated in a previous study establishing D-dimer as a marker of metastasis and tumour progression[123]. These findings have been reinforced by a recent retrospective study that showed an association between CTCs and elevated D-dimer levels in patients with advanced breast cancer[124]. Thus, D-dimer is not only a marker breast cancer progression, but also a prognostic marker as well as a marker of breast cancer relapse[125][126][127]. While a cut-off value greater than 500ng/ml is often taken as the probability of VTE in the general population, in cancer patients a value greater than 1440ng/ml is associated with an increased risk of VTE, and has been incorporated into a risk assessment model[128]. This author and his colleagues in a similar research calculated from a receptor operator curve (ROC) a sensitivity and specificity of D-dimer as a marker of lymph node involvement of 82.9% and 50% respectively, and a quantitative value of 1180ng/ml to indicate lymph node involvement[118]. Gochhait et al also

did a similar study, but did not give a quantitative value for lymph node involvement[129]. Fibrinogen is a known source of fibrin to tumour cells, has also been observed to be involved in the metastatic potentials of tumour cells and their intravascular survival through the prevention of NK cell mediated elimination[130]. Like D-dimer, fibrinogen is also associated with breast cancer progression. It is also associated with clinical stage, tumour size, tumour stage and lymph node involvement[131]. Cancer cells have been shown to form a fibrinogen-dependent bridge and transmigrate through the endothelium thus highlighting the metastatic potential of fibrinogen[132]. Elevated levels of fibrinogen have also been shown to be associated with overall survival in breast cancer patients; while it also serve as a predictor for clinical response in patients undergoing chemotherapy[133][134][135][136][137][138]. Elevated fibrinogen also impairs treatment response to trastuzumab, with fibrinogen values > 2.88 g/l being the cut-off. Izuegbuna et al also showed through a ROC that a fibrinogen value greater than 4.47g/l was associated with lymph node involvement[118].

8. The fibrinolytic pathway and breast cancer

Fibrinolysis is a normal physiological process in the body. Fibrinolysis is a regulated enzymatic process that involves the breakdown of the fibrin clot by proteins in order to prevent the intravascular accumulation and propagation of the clot which can lead to organ ischaemia and infarction. Thus, fibrinolysis is needed to achieve haemostatic balance. Fibrinolysis has been known to play a role in cancer for many years with evidence in the early 20th century showing that tissues from some malignant tumours have fibrinolytic properties. This fibrinolytic properties found in tumours have been primarily attributed to the plasminogen activators (PA) secreted by the tumours. These PA which function properly under normal physiological conditions are deregulated in different cancers. These PAs are proteases, and their inhibitors. They include the key protease plasmin, its precursor inactive plasminogen which is activated by the tissue- and urokinase-type plasminogen activators (tPA and uPA)[139]. The activation of plasminogen to plasmin by tPA or uPA lead to the degradation of the fibrin clot by plasmin into soluble fibrin degradation products (FDPs). Apart from this function, plasmin is also involved in the degradation of the extracellular matrices (ECM) to facilitate tissue remodeling or cell migration as well as activating growth factors. Thus, plasmin plays a key role during cancer invasion and metastasis. Plasmin activities are counterbalanced by its specific inhibitor, α2-antiplasmin, and a nonspecific protease inactivator, α2-macroglobulin. On the other hand, the activities of the plasminogen activators (tPA and uPA) are regulated by plasminogen activator inhibitor-1 (PAI-1) and -2 (PAI-2) and activated protein C inhibitor (PAI-3)[139][140][141]. It should be noted that certain proteases of the contact pathway of blood coagulation, plasma kallkrein (PK) and coagulation factor XIIa (fXIIa) can also activate plasminogen, albeit at lower efficacies compared to tPA or uPA. In addition to tissue remodeling and cell migration, the fibrinolytic system is heavily involved in the metastatic process in breast cancer and some other cancers; it plays a role in recruitment of inflammatory cells, modulation of cytokines, tumour cell growth and survival, angiogenesis, immune response and regulation of growth factors[142][143]. Over the years, certain components of the fibrinolytic system have been shown to be much involved in breast cancer progression. These include uPA, uPA receptor (uPAR), and PAI-1. While these three do not disrupt normal growth, studies have shown that homozygous deletion of the plasminogen (Plg–/–) gene in animals lead to fertility issues, growth retardation and reduced survival compared to those with an intact gene (Plg+/+)[144].

The tPA has been shown to be present in both normal and malignant tissues, uPA has been found mainly in malignancies, playing a major role in tissue remodeling. Thus, along with uPAR and PAI-1 are seen as effective prognostic and therapeutic targets in breast cancer. uPA is a 53-kDa serine protease initially expressed as a single chain inactive zymogen, pro-uPA, which undergo a two-step cleavage by other proteases (plasmin, cathepsins and human kallikrein type 2) to first form a two-chain high molecular weight uPA (HMW uPA), and further cleaved into a proteolytically-active low molecular weight uPA (LMW-uPA) with its plasminogen activator function intact. uPA comprise of three domains which are an amino-terminal domain, which contains the binding site for uPAR, a carboxy terminal sequence containing the catalytic site, and a kringle domain of a yet unknown function. uPA converts the zymogen plasminogen to its active form plasmin which is responsible for the degradation of many ECMs proteins including fibrin, laminin, fibronectin, and osteopontin. Plasmin also converts pro-MMPs to enzymatically active MMPs which are involved in ECM remodeling[145]. Plasmin can also cleave pro-uPA to create HMW-uPA promoting a positive feedback loop in the process. There is an overexpression uPA in tumour cells compared to normal cells where it plays an important role in tumour cells' invasion and migration. Increased levels of uPA are correlated with traditional prognostic factors such as tumour size, grade and CTCs; and it is also associated with a significant shorter OS and PFS in breast cancer patients[146][147][148].

For plasmin to aid cancer cell migration through ECM degradation, uPA must bind to uPAR to initiate the conversion of plasminogen to plasmin. The uPAR also known as CD87, is a member of the lymphatic antigen-6 superfamily. With a molecular weight of between 55 to 60kDa, it is a single-chain membrane glycoprotein receptor with about 313 amino acid residues. uPAR is made up of three domains, D1, D2 and D3 covalently linked to the outer layer of the cell membrane by a glysocylphosphatidylinositol (GPI) anchor. The three domains play a role in binding uPA to uPAR, while D2 and D3 are thought to be the ones involved in the interaction with other proteins. Apart from the conversion of plasminogen to plasmin, uPAR is involved alteration in cell adhesion and signaling by its interaction with various cell surface proteins. These include integrins (α5β1, α3β1, αvβ3 and αvβ5), vitronectin, G-protein coupled receptors (GPCRs), the receptor tyrosine kinases [epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR)], and very low-density lipoprotein receptor (VLDLR). These interactions lead to the activating of some signaling effectors such focal adhesion kinase (FAK) signaling, Ras/mitogen-activated protein kinase (MAPK), Ras-related C3 botulinum toxin substrate 1 (Rac1)/MAPK, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), and Janus-associated kinase 1 (JAK1) signaling pathways. Thus, triggering cellular responses such as cell migration, adhesion, epithelial–mesenchymal transition (EMT), proliferation and angiogenesis which are all associated with breast cancer progression. Under physiological conditions, uPAR is expressed at low levels, but can be higher under certain inflammatory conditions. However, it is overexpressed in many tumour tissues including breast cancer[149]. Because of its very low levels in healthy tissues and elevated level in tumours uPAR holds a prime position in cancer diagnosis and therapeutic monitoring. uPAR-based imaging strategies are being developed to ascertain cancer aggressiveness, and are currently undergoing clinical trials. The inhibition of uPAR expression has been shown to prevent tumour invasion and migration. In breast cancer, inhibition of uPAR alone or in combination with trastuzumab prevented the invasion and migration of different breast cancer cell lines[150][151]. In addition, uPAR have been linked to drug resistance in cancer cells. High expression of uPAR

has been shown to reduce the efficacy of tamoxifen in breast cancer patients[152]. Higher expression of uPAR was also associated with tamoxifen resistance in MCF-7 cell line, and doxorubicin and paclitaxel resistance in MDA-MB-231 cell line[153]. uPAR thus can be a predictive factor for response to therapy alongside a marker for breast cancer progression.

PAI-1 is a 45kDa serine protease inhibitor. It belongs to the serine protease inhibitor (SERPIN) protein family which also consists of PAI-2 and PAI-3 (protein C inhibitor), protease nexin-1, and neuroserpin. It is the principal inhibitor of both uPA and tPA. PAI-2 can also inhibit uPA, but more slowly. High expression of PAI-1 is reported to be associated with metastasis, but ironically PAI-2 is protective[154]. Apart from its role in preventing plasminogen activation, PAI-1 is also known to bind to vitronectin and low-density lipoprotein receptor-related protein-1 (LRP1), augmenting cell motility and migration. The LRP1 helps the internalization of the uPA-PAI-1-uPAR complex via a clathrin-mediated endocytosis. A high level of the uPA-PAI-1 heteromerization is known to promote breast cancer progression as well as predict treatment response and survival[155]. Many studies have shown that uPA/PAI-1 have a good predictive value to forecast response to some chemotherapies in breast cancer[156][157][158]. Though, it was shown to have intermediate level of evidence (LOE-II) with regards to response to cyclophosphamide, methrotrexate, flouracil regimen (CMF)[156]. In pN0 patients, uPA/PAI-1 reached the highest level of evidence (LOE I) for the prognostic value of disease-free survival at 10 years; and are the only haemostatic breast cancer markers to reach LOE-1. Thus, the American Society for Clinical Oncology (ASCO) in 2007 recommended the use of uPA and PAI-1 ELISA for assessment of the risk of reoccurrence in breast cancer patients[159][160]. A large meta-analysis by the Receptor and Biomarker Group of the European Organization for Research and Treatment of Cancer (EORTC) also validated the prognostic role that uPA and PAI-1play in breast cancer[161].

Due to their important role in breast cancer progression, uPA, uPAR and PAI-1are seen as attractive therapeutic targets in the management of breast cancer. A number of small molecules and other novel inhibitors have been developed, and some are undergoing clinical trials[162][163][164]. A recent study by Wrzeszcz et al reported that a lower baseline plasma concentration of t-PA antigen and higher PAI-1 activity might be strong predictors for distant metastases and independent prognostic markers in breast cancer patients[165].

9. Endothelial cells and breast cancer.

Endothelial cells (ECs) form a single cell layer that lines the blood vessels. They play a key role in vascular haemostasis, most especially in thrombosis formation, fibrinolysis, and vascular remodeling. The normal endothelium is both anticoagulant and antithrombotic, while a compromised one shifts the balance to a procoagulant/prothrombotic phenotype. The normal endothelial cell is involved in maintaining haemostasis through the secretion of a number of substances that are important in blood coagulation. Some of these substances include prostacyclin (PGI2) and nitric oxide (NO) which prevents the aggregation of platelets, thus, limiting the intravascular extension of thrombus formation. It also expresses different anticoagulants such as tissue factor pathway inhibitor (TFPI), thrombomodulin, endothelial protein C receptor (EPCR), and heparin-like proteoglycans. Besides its action on platelets, the endothelial cell when activated is also involved in thrombin signaling through PAR-1 that is present on its surface, thus playing a role in the coagulation. In reciprocation, thrombin causes the release of VWF, P-selectin presentation at the plasma membrane, and production of

platelet activating factor (PAF) by the endothelial cells. The endothelium also plays a role in fibrinolysis by the release of tPA and uPA. To balance things out, it also constitutively secrete PAI-1. In like manner, the endothelial cells do not synthesize TF, but when activated by thrombin or other inflammatory mediators, it synthesizes it. A substance like PAF is a powerful platelet activator which causes platelet adhesion to endothelial cells. In the same vein, VWF which is stored in the Weibel-Palade bodies can be mobilized from the endothelial cells for the binding of platelets to exposed extracellular matrix components when the vessel wall is damaged. In summary, the endothelial cells are involved with other markers that play a role in breast cancer progression.

Endothelial cells from breast cancer cells have been shown to be differ significantly, both phenotypically and functionally from endothelial cells from normal tissues[166]. The tumour microenvironment is known to influence the EC phenotype, and aids in tumour cell proliferation and migration. Breast cancer cells are known to mediate endothelial cell activation, as well as promote a tumoural niche that supports survival, stemness and metastasis. In vitro studies show that endothelial cells Jagged1 promote breast cancer growth through a notch-dependent activation[167]. Markers of endothelial cell activation have also been implicated in breast cancer growth and progression. The VWF which is a large multimeric plasma glycoprotein is known to play an important role in haemostasis; besides, it is primarily secreted by endothelial cells. Beyond its normal role in haemostasis, it is reportedly involved in inflammation, angiogenesis and cancer metastasis. Increased level of VWF antigen (VWF:Ag) have been reported in various cancers including breast cancer; moreover, significantly higher levels were detected in metastatic cancers[168]. It has also been shown to be a thrombotic risk in breast cancer. Breast cancer progression and metastasis have been linked to VWF. Pioneering research done in the 1980s showed that antibodies targeting VWF caused a lower interaction between platelets and cancer cells in vitro, and reduced metastatic potentials in mice[169]. However, VWF activities may differ depending on cancer types. In breast cancer, Dhami et al reported breast cancer mediate EC activation which cause the release of endothelial markers stored in Weibel-Palade bodies (VWF, osteoprotegerin and angiopoietin-2) as well as induce angiogenesis and transendothelial migration; including survival[170]. VWF is also reported to shield metastatic cells from chemotherapy. Interestingly, VWF do have anti-metastatic properties which can be exploited in breast cancer therapy[171]. Pre- surgical levels of VWF in breast cancer patients have also been shown to be correlated with tumour differentiation, grade and a predictor of disease relapse. Thus, VWF can a predictive factor in breast cancer[172].

PAF, another mediator synthesized by the endothelial cell (also synthesized by neutrophils, platelets and monocytes) is known to play a role in angiogenesis, thrombosis, carcinogenesis, and metastasis. In breast cancer, more malignant cells have a greater ability to synthesize and release PAF, metastasize, and also express more PAF-R on their membranes. PAF may be involved in breast cancer initiation as well as promotion by enhancing the migratory ability of cancer cells mediated via phosphoinositide 3-kinase and/or the Jun N-terminal kinase pathway, which is independent of the mitogen-activated protein kinase pathway[173].

10. Conclusion

The last few decades have witnessed great strides in our understanding of haemostatic markers and breast cancer. The

activation of the haemostatic milieu is a common occurrence in cancers generally, and many of the haemostatic markers are known to be involved in the proliferation, and migration of cancer cells. They usually represent an increased tumour burden and poor prognosis. While several clinical studies have shown that haemostatic markers are suitable in breast cancer progression, validation remains an issue. Till date, only the tumour-associated uPA and PAI-1 have been validated as prognostic markers for breast cancer. Others, such as fibrinogen and D-dimer have shown great promise in breast cancer prognosis. The D-dimer has been validated for risk of thrombosis in cancer patients, and the possible need of thromboprophylaxis. These developments have shown that haemostatic markers can have potentials in diagnostics and therapeutics. Already, an antibody conjugate has been developed against TF, and some therapies are in clinical development against uPA and uPAR. There is hope that the use of some haemostatic markers after due validation can serve as diagnostic and therapeutic tools in cancers generally, leading to a reduction in morbidity and mortality in these malignancies, including breast cancer. The HYPERCAN (“HYPERcoagulation and CANcer”) an ongoing prospective, multicenter, observational study which started in 2012 with the objective of assessing if the occurrence of a hypercoagulable state may be predictive of cancer diagnosis in healthy individuals, or may be predictive of disease recurrence, clinical progression and thrombosis in cancer patients. Several biomarkers are being evaluated including D-dimer, fibrinogen, thrombin generation assay, TF, prothrombin fragment 1 + 2 (F1 + 2), MP procoagulant activity, protein C, protein S, t-PA, PAI-1, FVIII and FXIII. It is hoped studies like this would generate some useful results. For the time being, more clinical studies and analysis will be required to validate the haemostatic biomarkers and get them into the clinics.

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Competing interests

The authors declare no competing interests.

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