Received: 27 October 2021 | Accepted: 17 February 2022

DOI: 10.1111/jth.15680

REVIEW ARTICLE

Thrombomodulin and pregnancy in the limelight: Insights into the therapeutic aspect of thrombomodulin in pregnancy complications

Hiroko Oda | Takeshi Nagamatsu | Yutaka Osuga

Department of Obstetrics and Gynecology, Faculty of Medicine, The University of Tokyo, Bunkyo-ku, Japan

Correspondence
Takeshi Nagamatsu, Department of Obstetrics and Gynecology, The University of Tokyo, 7-3-1 Hongo, 113-8655 Bunkyo-ku, Tokyo, Japan.
Email: tnag.tky@gmail.com

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 15H04979 and 18H02943

Abstract

Background: Thrombomodulin (TM) is a transmembrane glycoprotein expressed on the endothelial cell functioning as a cofactor in the anticoagulation system. However, aside from anticoagulation, recent studies have revealed its multiple organ protective roles such as anti-inflammation, angiogenesis, and cell proliferation, which may redefine the function of TM. Although TM is predominantly expressed on placental trophoblasts, the physiological role of TM during pregnancy remains unclear. Because the understanding of TM function has drastically progressed, these new discoveries shed light on the unknown activities of placental TM. Moreover, the clinical application of recombinant TM (rTM) has opened the possibility of TM as a therapeutic target for pregnancy complications.

Objectives: Here, we comprehensively review the studies elucidating the role of TM during pregnancy from both classic and newly discovered perspectives, and seek for its potential as a therapeutic target for pregnancy complications.

Methods: Basic research using trophoblast cells and transgenic mice, as well as cohort studies of inherited TM deficiency and clinical trials of rTM were summarized, which led us to further discuss the clinical application of rTM as a novel therapeutic for pregnancy complications.

Results and Conclusion: Accumulating evidence suggest the relevance of placental TM deficiency in pregnancy complications such as miscarriage, fetal growth restriction, and preeclampsia. Most importantly, promising results in animal studies and clinical trials further assure the possibility of rTM as an optimal therapeutic for such conditions. The therapeutic potential of TM raised throughout this review could drastically change the clinical approach to pregnancy complication and improve maternal outcomes.

KEYWORDS
placental homeostasis, pregnancy, thrombomodulin, trophoblast cell

Manuscript Handled by: Roger Preston

Final decision: Roger Preston, 17 February 2022

1 | INTRODUCTION

Thrombomodulin (TM) is a transmembrane glycoprotein localized on the vascular endothelial cells. Because TM integrates various physiological processes by regulating thrombosis, inflammation, and cell proliferation in a host-protective manner, it is considered to play a crucial role in organ homeostasis. TM is abundantly synthesized by trophoblast cells; thus, it is speculated that TM may also play a role in the maintenance of placental function. Since TM was initially isolated from human placenta in the 1980s, its physiological functions have been intensely studied. Given that thrombophilia is a well-known risk factor for adverse pregnancy outcomes, anticoagulative roles of TM involving protein C (PC) were particularly focused in the 1990s. Although, recently, studies have shed light to its diverse properties beyond anticoagulation, exerted directly by TM itself, and by interaction with other cell surface receptors or coworking molecular complexes, regulating proinflammatory cytokines and complement activities. These discoveries in the past decade have become a breakthrough to further understand the physiological role of TM during pregnancy. More importantly, these newly elucidated properties may have potential therapeutic applications for the management of pregnancy complications, such as miscarriage, fetal growth restriction (FGR), and preeclampsia (PE). In this review, we summarize the involvement of TM during pregnancy, including the relevance of its recently reported diverse properties, as well as its potential as a therapeutic target for pregnancy complications.

2 | MOLECULAR PROPERTIES OF TM

2.1 | Structure and functions

Thrombomodulin is a 557-amino acid type-1 transmembrane glycoprotein comprising five structural domains (Figure 1).[1,2] The N-terminal C-type Lectin-like domain (D1) is followed by six epidermal growth factor (EGF)-like repeats (D2), serine/threonine-rich domain (D3), and the transmembrane region (D4) with a short cytoplasmic tail (D5). The N-terminal region (D1) consists of a domain with homology to C-type lectin family members.[3] D1 is responsible for anti-inflammatory activity. A proinflammatory cytokine, high mobility group Box1 (HMGB1), is cleaved and deactivated by binding to this region.[4] It also interacts with Lewis^y antigen, which regulates leukocyte trafficking.[5] D2 consists of six EFG-like repeats, forming a long stalk of the TM structure. D2 is responsible for anticoagulation, which is the characteristic property of TM. Thrombin shows high affinity to the EGF5-6 repeats, forming a thrombin-TM complex that activates PC (APC).[6] Although EGF5-6 accelerates APC production, EGF4-6 repeat is also capable of PC activation independent of thrombin. The EGF3-6 repeat transforms thrombin-activatable fibrinolysis inhibitor (TAFI) into its activated form aTAFI, which downregulates fibrinolysis. D3 has the potential for O-linked glycosylation and chondroitin sulfate attachment. D3 affects mitogenesis by upregulating matrix metalloproteases (MMPs) and plasminogen activators.[7] A single-pass transmembrane structure (D4) followed by a short cytoplasmic tail (D5) is the fourth and fifth domain of TM, respectively, practically attaching the molecule to the cell surface. D4 is the target site for TM cleavage upon endothelial cell damage,[8] whereas D5 regulates cytoskeletal organization via the adaptor protein ezrin.[9]

Thrombomodulin is a transmembrane protein expressed on the endothelium, forming the luminal lining of blood vessels. In conditions underlying endothelial damage, TMs are proteolytically cleaved from the membrane, resulting in the elevation of circulating TM fragments in the blood flow recognized as soluble TM (sTM).[10] Although the mechanisms of TM cleavage remain unknown, leukocyte-induced proteases may play a role in its proteolysis.[8] sTM is recognized as a biological marker of endothelial dysfunction.[11] Patients with disorders that underlie endothelial damage, such as sepsis, disseminated intravascular coagulation (DIC), and PE show elevated serum sTM levels.[11,12]

2.2 | Anticoagulant activities: classic roles of TM

The anticoagulative properties of TM are crucial for the homeostasis of mammalian circulation. Gene-deficient mice lacking vascular endothelial TM develop hypercoagulopathy, resulting in lethal thromboembolism.[13] Thrombin has a high affinity for TM, forming the thrombin-TM complex.[14] Once bound to the EGF5-6 repeat, the function of thrombin is converted from its original procoagulant status to anticoagulation.[6] TM inhibits thrombin activities including fibrinogen cleavage, interaction with protease-activated receptors (PAR) and activation of factors V and XIII. Most importantly, thrombin-TM complex serves as a cofactor in APC production. The PC activation is significantly accelerated in the presence of thrombin-TM complex. APC then combines with protein S(PS), which degrades FVa and FVIIIa, and thereby suppresses further thrombin generation. TM- and TM-PC-mediated pathways have long been proven to be relevant to the clinical occurrence of thrombosis. Patients with polymorphisms of PC, PS, Factor V-Leiden (FVL), and TM are at high risk of thromboembolism.[15] Another antithrombotic function of TM is the activation of TAFI by EGF3-6 repeats.[16] Activated TAFI cleaves C-terminal lysine residues of degraded fibrin, which abrogates its function as a cofactor in plasminogen-plasmin conversion and thereby modulates fibrinolysis.[17] Although the activation processes of TAFI and PC share the same EGF region of TM, these pathways do not affect each other.[18] These two seemingly identical anticoagulative properties of TM are independently regulated in the homeostasis of circulation, which is further clarified by their downstream activities under inflammatory conditions.

2.3 | Anti-inflammatory activities: newly discovered roles of TM

In the past decade, studies revealed the anti-inflammatory properties of TM, independent of its role in coagulation. Mice with TM

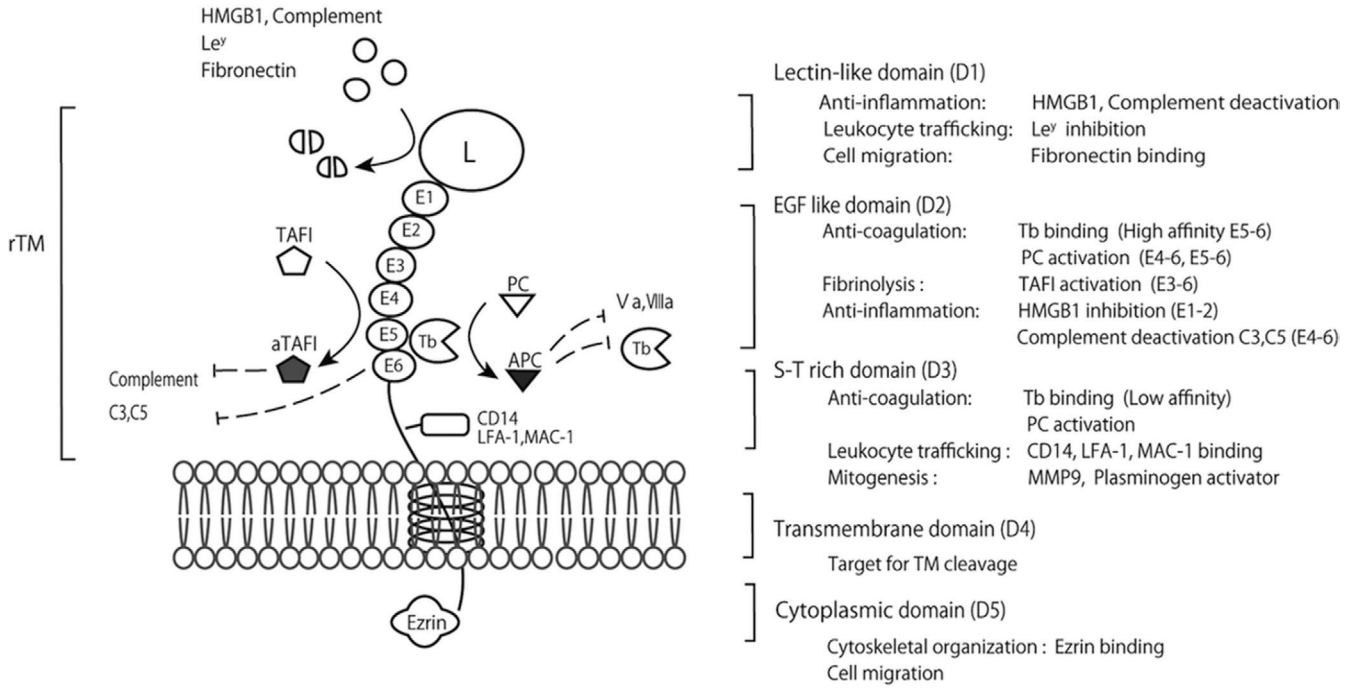


FIGURE 1 Structure and function of TM. Thrombomodulin (TM) comprises five structure domain. N-terminal C-type lectin-like domain (D1) is followed by six EGF-like domain (D2), serine/threonine (ST) rich domain (D3), and the transmembrane domain (D4) with a cytoplasmic domain (D5). Lectin-like domain (D1) regulates anti-inflammation by binding high mobility group box1 (HMGB1) and complements. It also interacts with Lewis^y (Le^y) antigen and fibronectin, modulating leukocyte trafficking and cell migration. EGF domain (D2) is in charge of anticoagulation. Thrombin (Tb) shows high affinity to D2, forming a Tb-TM complex and activating protein C (PC). D2 activates thrombin activatable fibrinolysis inhibitor (TAFI), which downregulates fibrinolysis. HMGB1 and complement deactivation occurs within D2 as well. The ST-rich region (D3) affects mitogenesis by upregulating MMP and plasminogen activators. It interacts with CD14 and adhesion molecule LFA-1 and MAC-1 regulating leukocyte migration. Although the affinity is low, Tb binds to D3, enhancing PC activation. Transmembrane domain (D4) is the target site for TM cleavage upon cell damage. Cytoplasmic domain (D5) regulates cytoskeletal organization and cell migration via adaptor protein ezrin. Recombinant TM (rTM) comprises functionally active extracellular D1-3

gene mutations exhibit an increased susceptibility to sepsis, demonstrating that TM is a regulator of inflammatory responses.[19] Anti-inflammatory functions of TM are characterized into two categories: APC-dependent and APC-independent mechanisms. APC generation is significantly accelerated by TM.[6] APC-dependent anti-inflammatory mechanisms are exerted by APC itself and TM-APC complex interacting with another cell-surface receptor regulated signaling. Dependent on the EGF 4–6 repeats of TM for its generation, APC is known to suppress the synthesis of nuclear factor-kappa B (NF-κB), which in turn decreases its nuclear translocation and downregulates proinflammatory cytokine production.[20] These activities rely on the process of APC forming a complex with endothelial cell protein C receptor (EPCR) and PAR-1, which initiates G protein-mediated cytoplasmic anti-inflammatory signaling.[21,22] Furthermore, APC directly interferes with neutrophil inhibiting its proinflammatory activities and extracellular trap release independent of NF-κB.[23]

Suppression of complements is one of the major APC-independent anti-inflammatory functions of TM.[24,25] Because aTAFI serves as a suppressor of complements C3a and C5a,[24] the EGF domain of TM plays a role in the regulation of complement-mediated inflammatory reactions by activating TAFI. The Lectin-like domain serves as a cofactor for factor I and H in the deactivation process of C3 convertase; thus, it is also involved in complement activity.[25] Indeed, lectin-like domain deficiency enhance complement activation in animal models of inflammatory arthritis, and diabetic kidney damage.[26,27] The Lectin-like domain is responsible for the other APC-independent anti-inflammatory property of TM; the cleavage of HMGB1.[4] HMGB1 is a DNA-binding nuclear protein that serves as a danger signal when secreted into the extracellular space upon cell damage.[28] HMGB1 binds to the cell surface receptors, RAGE, and Toll-like receptors, thereby exaggerating secondary inflammatory reactions.[28] TM prevents proinflammatory signaling by binding extracellular HMGB1 to the Lectin-like domain and partially to the EFG-like domain.[4,29] Indeed, transgenic mice lacking Lectin-like domain show high mortality rates in endotoxin-induced lung damage and arthritis.[26,30] TM negatively regulates NF-κB transcription via HMGB1, independent of APC. Inhibition of HMGB1 is an anti-inflammatory function of TM that consequently inactivates Toll-like receptors and RAGE.[4,28] These cell-surface receptors are known to upregulate NF-κB transcription, thus, TM plays a suppressive role in NF-κB activity.

Multiple roles played by each TM domain overlap and crosstalk by sharing the same TM structure and cofactors, but they mostly do

not affect each other. More importantly, there is always a backup player for these physiological pathways within the same TM molecule; thus, its function may be secured even when partial molecular damage occurs. TM is indeed, a well-organized molecule, capable and responsible for controlling complex biological systems.

3 | TM AND PREGNANCY

3.1 | Genetic mutation studies in mice revealing TM functions during pregnancy

A number of transgenic mice with total or partial TM gene mutations have been studied to elucidate its function in placental development (Table 1).[31–33] In rodent embryos, TM is detected in parietal endoderm cells from embryonic day (E)7.5.[34] By E10.5, TM is found in fetal vascular organs playing a role in organogenesis.[35] Complete loss of TM causes total embryonic lethality in the early stage of pregnancy (~E8.5).[36] Because fetal death occurs before the development of the cardiovascular system, these results imply the necessity of TM in early placentation process. This implication was supported by placenta-restricted TM reconstitution in TM-null mice.[32] TM-null embryos with placenta-restricted TM expression developed normal morphogenesis during midgestational period. However, they presented signs of organ hemorrhage between E12.5 and 16.5, causing intrauterine death. In transgenic mice lacking TM on vascular endothelium, 40% of the TM-null embryos retrieved at E10.5, presenting placental thrombosis.[13] The remaining 60% were born with normal morphogenesis, suggesting that TM on trophoblast cells, not endothelial cells, is required for placentation. Furthermore, TM may be required for the maintenance of fetal organ homeostasis rather than organogenesis.[13] Most recently, Mens et al. generated a TM-null mice using tissue selective gene ablation via Meox2Cre-transgene.[37] Selective preservation of TM in placenta and yolk sac enabled the TM-null fetus to survive to full term. Half of these complete-TM deficient pups survived until adulthood without severe systemic thrombosis or consumptive coagulopathy.[37] However, when Meox2Cre-TM null females were mated with wild-type males, carrying normal placentas and embryos, all of the dams ended in lethal distress between 11pc to term, exhibiting major maternal hemorrhage in the uterus and lungs.[37] Given these results, it could be speculated that placenta-restricted TM is sufficient for placental development and intrauterine fetal growth. TM-null mice may suffer systemic thrombosis but are capable of surviving their adulthood; however, systemic TM is necessary to tolerate maternal physiological changes induced by pregnancy.

Early lethality of TM-null embryos was not associated with the severity of pathological placental thrombosis or fibrin disposition. Instead, they were related to the severity of apoptotic cell death and proliferation failure of trophoblast cells, which was identical to the placentas obtained from mothers with FVL mutation.[38] FVL mutation is a known risk factor for adverse pregnancy outcomes, characterized by resistance to APC activities resulting in procoagulant status with overly produced thrombin. However, the EGF domain of TM that is a major generator of APC, is not responsible for fetal development. This was shown by the TM^{pro} mice exhibiting EGF4-5 interdomain loop mutations and presenting diminished placental thrombin-dependent APC generation. TM^{pro} embryo carried by normal TM-expressing dams fully survived without defect, even with the diminished APC activity in the placenta.[39] Because this mutation replicates the oxidative damage to TM,[40] these results could also be implicated that the TM function necessary for fetal growth, is preserved even under high oxidative stress within the uteroplacental unit. In contrast, when TM^{pro} embryos are carried by FVL dams, fetuses show growth arrest by E9.5 and intrauterine death by E10.5[38] Because homozygous FVL mice exhibit normal fertility and fetal growth, these studies suggest that the TM-mediated APC defect in the fetal side may not be a direct cause but a predominant risk factor for fetal lethality in maternal prothrombotic status. These animal studies suggest that the interaction of placental trophoblast-TM loss and maternal prothrombotic background could be a risk factor of pregnancy complications.

The pathological similarities in the placenta of $TM^{-/-}$ and FVL mutation, provoked the hypothesis that the activated coagulation factors may disturb placental development.[41] Administration of anticoagulants or genetic fibrinogen reduction delayed the resorption of TM-null embryos; however, it did not attenuate fetal growth defects.[41] TM-null embryos with impaired TF activity survive without defects.[41] TF generates fibrin disposition and fibrin degradation products (FDP), which is known to induce cell apoptosis. Indeed, *in vitro* and *in vivo* studies revealed that FDP induce cell death, and thrombin limits cell proliferation in trophoblasts.[41] Because FDP and thrombin are normally abrogated by TM, these results indicate that TM is involved in the survival mechanism of trophoblast cells via thrombin and fibrin disposition.[42] Transgenic mice with EPCR deficiency show similar phenotype with TM-null mice. However, EPCR null embryos survive until E10.5 with severe placental thrombosis, which is considered the direct cause of embryonic lethality. Because EPCR is less relevant to the process of FPD abrogation, the histological differences between TM and EPCR null placentas could be related to the amount of FDP accumulated in the placental tissue affecting trophoblast cell survival.[43]

Anti-inflammatory effects of TM have been intensely studied in the 2000s. The fetus of transgenic mice with reduced TM-lectin-like domain, a regulator of inflammatory responses, survived with no growth defects.[30] Cytoplasmic domain, regulating cell migration and cytoskeletal organization, was shown to be irrelevant to embryonic lethality as well.[33]

Genetic depletion of PAR-1 and PAR-2, the coworking receptors involved in inflammatory signaling, did not rescue TM-null embryos.[44] However, the ablation of platelets or PAR-4, the receptor mainly expressed on platelets, fully restored the development of TM-null fetuses and rescued the TM^{pro} embryos carried by dams with FVL mutation.[38,44] These studies imply that the proinflammatory role of platelet and PAR-4 could be the underlying mechanism of fetal death in TM-deficient embryos.

TABLE 1 Genetic mutation studies in mice revealing TM functions during pregnancy

Year	Author	Gene mutation	Phenotypic alteration	Fetal outcome	Summary
1995	Healy et al.[36]	$TM^{-/-}$	Embryo with total TM deficiency	<E8.5 fetal death	Maternal-embryonic interaction failure caused by total absence of TM in parietal yolk sac, results in early embryonic lethality
		$TM^{+/-}$	Embryo with partially disabled TM function (50% reduction)	Survive full term	Partial reduction of TM expression does not affect fetal lethality
1998	Weiler et al.[39]	$TM^{+/-}$ + $tPA^{-/-}$	Increased placental fibrin deposition in $TM^{+/-}$ embryos	Survive full term	Increased fibrin disposition subsequent to enhanced coagulation is not responsible for embryonic lethality in TM^{-} mice.
		$TM^{pro/pro}$	TM EGF4-5 domain deficiency (1000-fold reduced PC activation)	Survive full term	EGF4-5 domain is not responsible for early embryonic lethality.
		$TM^{-/pro}$	TM EGF4-5 domain deficiency (2000-fold reduced PC activation)	Survive full term	Embryonic development is unrelated to APC formation or thrombin inhibiting activity by EGF domain
1999	Conway et al.[33]	$TM^{cyt/cyt}$	TM cytoplasmic domain deficiency	Survive full term	Cytoplasmic domain is not responsible for TM^{-} embryonic lethality
2001	Isermann et al.[13]	$TM^{Lox-/Lox-}$	Loss of TM expressed on the endothelium	60% full-term survival, 40% fetal death by E10.5	Loss of vascular TM may inhibit placental blood vessels invading into the myometrium, which could induce placental dysfunction
2001	Isermann et al.[32]	$TM^{-/-}$ + $TM^{lacZ/lacZ}$	Placenta restricted TM expression in $TM^{-/-}$ embryo	E12.5–16.5 fetal death <E10.5 growth arrest	TM loss in embryonic blood vessels caused consumptive coagulopathy and fetal growth arrest
2002	Conway et al[30]	$TM^{Led/Led}$	Embryos with 20% reduction of TM Lectin-like domain	Survive full term	Partial reduction of Lectin-like domain may not affect fetal growth or lethality
2003	Isermann et al.[41]	$TM^{-/-}$ + heparin/ warfarin	$TM^{-/-}$ treated with Heparin or Warfarin	>9.5 dpc fetal survival, growth arrest <dpc 8.5	Pharmacological anti-coagulation delays embryonic lethality
		$TM^{-/-}$ + tissue factor defect	Embryo lacking TM and tissue factor activity	>10.5 dpc fetal survival, fetal defect at E9.5	TF <1% completely rescues TM^{-}embryos from early lethality. TF deficiency directly cause placental/ fetal defect
		$TM^{-/-}$ + maternal and embryo $Fib^{-/-}$	$Fibrinogen^{-}$ dam with embryos lacking TM and fibrinogen	>10.5 dpc fetal survival, growth arrest at E8.5	Maternal fibrinogen gene deficiency prolonged TM^{-} fetal survival
		$TM^{-/-}$ + maternal $Fib^{-/-}$	$Fibrinogen^{-}$ dam with embryos lacking TM	>10.5 dpc fetal survival, growth arrest at E8.5	Maternal fibrinolysis inhibition, rather than fetal, preserved fetal death in TM^{-}embryo.
		$TM^{-/-}$ + embryo $Fib^{-/-}$	Embryos lacking TM and fibrinogen	<9.5 dpc fetal death	

TABLE 1 (Continued)

Year	Author	Gene mutation	Phenotypic alteration	Fetal outcome	Summary
2007	Sood et al.[38]	$Fv^{Q/Q}$ + $TM^{pro/pro}$ embryo	Maternal APC resistance from FVL polymorphism carrying embryos with reduced APC activity	<E9.5 growths arrest, <E10.5 fetal death	TM mediated APC defect in the fetal side is a predominant risk factor for fetal lethality in maternal prothrombotic status.
		$Fv^{Q/Q}$ + Plt depletion (E7.5~) + $TM^{pro/pro}$ embryo	Immunodepletion of maternal platelets in $Fv^{Q/Q}$ dams carrying TMpro embryos from E7.5	Survive full term	Maternal platelet mediated insult to the placenta occurs as early as E7.5
		$Fv^{Q/Q}$ + Plt depletion (E9.5~) + $TM^{pro/pro}$ embryo	Immunodepletion of maternal platelets in $Fv^{Q/Q}$ dams carrying $TM^{pro/pro}$ embryos from E9.5	<E10.5 fetal death	
		$Fv^{Q/Q}$+$Par4^{-/-}$ + $TM^{pro/pro}$ embryo	Maternal PAR4 deficiency in $Fv^{Q/Q}$ dams carrying $TM^{pro/pro}$ embryos	Survive full term	Maternal $Par4^{-/-}$ overcomes fetal/placental developmental block due to APC resistance
2008	Sood et al.[44]	$TM^{-/-}$ + embryo $Par1^{-}$/$Par2^{-}$	$TM^{-/-}$with PAR1 or PAR2 deficient embryo	<E10.5 fetal death, growth arrest at E8.5	PAR1 and PAR2 inhibition does not recover fetal lethality of TM-embryo.
		$TM^{-/-}$ + $Par4^{-}$	$TM^{-/-}$ with maternal PAR4 deficiency	>E9.5 fetal survival	PAR4 inhibition prolongs survival of TM^{-} embryo
		$TM^{-/-}$ + $Nfe2^{-/-}$	$TM^{-/-}$ with maternal platelet deficiency	>E9.5 fetal survival	Maternal platelet deficiency prolongs survival of TM^{-} embryo
		$TM^{-/-}$+$C5^{-}$,$C3^{-}$maternal and embryo	$TM^{-/-}$with complement deficiency in dam and embryo	<E8.5 fetal death	Complement activities does not a cause fetal death in TM^{-}embryo
		$TM^{-/-}$ + LMWH	$TM^{-/-}$with LMWH administration	<E8.5 fetal death	LMWH does not preserve fetal death of TM^{-}embryo
2017	Mens et al.[37]	Meox2Cre-TM^{loxP}	$TM^{-/-}$ embryo with preserved TM function in the extraembryonic ectoderm and primitive endoderm	Survive full term	Placenta-selective preservation of TM is sufficient for embryonic growth in TM^{-} mice. 50% of Meox2Cre-TM null pups died of birth-induced thrombohemorrhagic complication
		Meox2Cre-TM^{-}dam+ Pregnancy	Pregnancy of TM null dams grown from Meox2Cre-TM^{-} embryos	Maternal death	Pregnancy induces maternal death in Meox2Cre-TM^{-} dams, causing uterus and lung hemorrhage.

Abbreviation: TM, thrombomodulin.

Platelet is a major source of HMGB1-mediating inflammation.[45] Considering our previous study revealing the function of HMGB1, regulating placental angiogenesis via HIF,[46] it could be speculated that maternal platelet or PAR-4 on platelets may play a role in HMGB1 accumulation to the placenta that inhibits placental angiogenesis, and consequently induce embryonic lethality.

There is a rising possibility that TM is responsible for the trophoblast cell survival and inflammatory regulation rather than antithrombosis during placental development. Because the pattern of TM expression is conserved in both mice and humans, these studies provide insight into the function of TM in human pregnancy. However, the physiological functions and domains of TM are not in one-to-one correspondence. It is important to consider that the results of domain-specific gene depletion studies may not fully reveal the physiological function of TM in pregnancy.

3.2 | Human TM polymorphism and pregnancy outcomes

Thrombophilia is associated with adverse pregnancy outcomes.[47] Genetic APC resistance from FVL mutation is a well-known risk factor for fetal death.[47] Because TM is a key player in coagulation, mothers with genetically impaired TM were considered at high risk for maternal complications. Although several TM gene mutations have been identified so far, the relationship between adverse pregnancy outcomes and TM polymorphism is under debate.[48–56] Some TM gene mutations are likely to affect pregnancy; however, studies did not reach statistical significance (Table 2). This could be explained by several factors. First, TM mutations are extremely rare in humans. Because TM function is incompatible with fetal development, it is possible that fetuses with major alterations in TM may not survive.[36] Second, the expression of TM genetically differs according to the ethnicity. Maternal sTM level is not the same in mothers of different ethnicities.[51] Thus, the statistical relevance of TM polymorphisms in pregnancy outcomes may vary between populations. Considering that TM mutations are rare and their association with pregnancy complication is unclear, general screening during pregnancy would not be clinically beneficial.

3.3 | Function of TM during pregnancy

Thrombomodulin plays a different role in each gestational and maternal organ depending on gestational age (Figure 2). Because the key for successful pregnancy is the regulation of coagulation and inflammation that interfere with each other, TM may act as a bridging player shared by these pathways. Proinflammatory factors are produced in the uteroplacental unit and flow into the maternal circulation, which makes pregnancy a state of systemic inflammation. Maternal vascular TM plays a protective role against these inflammatory products to prevent endothelial dysfunction and organ damage. Intense TM expression has been detected in the gestational tissues. In the initial stage of pregnancy, TM is involved in embryonic implantation by maintaining the cytoskeletal organization of endometrial cells.[57] TM is identified on the endometrium, which is modified into decidua during placentation. Endometrial TM connects to actin filaments via bridging protein ezrin, which is responsible for cell-to-cell adhesion and cytoskeletal development.[9] TM contributes to the maintenance of endometrial barrier integrity that determines the receptivity of embryos.[57]

Because TM was first isolated from the placenta as "fetomodulin",[58] TM is detected on the placental vascular endothelium, syncytiotrophoblast, myometrium, and fetal membrane.[2,59,60] Syncytiotrophoblast is functionally equivalent to the vascular endothelium, forming the lining of the intervillous space. Considering the function of TM in maintaining the interface of circulating blood flow and organs, it is not surprising that TM is found specifically in syncytiotrophoblast, but not in cytotrophoblasts nor stromal cells present in the inner layer of the villi.[61] The expression of TM in the placental vascular endothelium increases with gestational age, most likely coordinating with the growing need for blood supply.[59,61] TM is also involved in the homeostasis of placental trophoblasts by regulating cell apoptosis and proliferation cycle.[41,42] Our current study revealed the relevance of TM in the placental hypoxic stress coping mechanism by regulation of HIF signaling. Placental TM reduces HIF1a activity by inhibiting HMGB1 and enhancing placental growth factor expression, which promotes angiogenesis.[46] TM plays a role not only in the development of sufficient uteroplacental circulation, but also in the maintenance of placental cell turnover, functional homeostasis, and stress coping mechanisms (Figure 3).

In normal pregnancy, serum sTM levels continuously increase throughout gestation and rapidly decrease after delivery.[62] Considering the intense expression of TM in trophoblast cells, it is reasonable to assume that the placenta is the source of excessive maternal sTM. It is acknowledged that sTM levels significantly increase in preeclamptic mothers.[63–66] Although the amount is relatively low, sTM is also found in amniotic fluid.[67] sTM detected in amniotic fluid is most likely nonfunctional, secreted from the fetus via urine or bronchial excretion. TM is essential for fetal organogenesis. Indeed, fetal serum sTM levels are several times higher than that of their mothers.[67] Enhanced sTM in fetal circulation could reflect the activity of TM for fetal organ development. However, the precise function of TM in fetal development remains to be clarified. Although its function is largely unknown, these findings imply that TM is deeply involved in the physiology of pregnancy.

4 | THE ROLE OF TM IN PREGNANCY COMPLICATIONS

Regarding its possible relevance during pregnancy, we have reviewed the studies elucidating the role of TM in pregnancy complications.

TABLE 2 The relationship between adverse pregnancy outcomes and TM polymorphism

Year	Author	Gene mutation	Pregnancy complication	Study population	Study design	Relevance	Mutation frequency (% control group)
2007	Kaare et al.[68]	TM, c.1418C>T (EGF region)	Recurrent miscarriage	Finland	Cohort	N	8/191
		TM, c.1728+23_+40del	Recurrent miscarriage	Finland	Cohort	N	0/191
2013	Cao et al.[48]	TM,c.C1418T polymorphism (EGF region)	Recurrent pregnancy loss (<20 wk)	China	Case control	S	63/169
2001	Franchi et al.[49]	TM, c.201-G>A (promoter region)	Late pregnancy loss (>20 wk), Placental thrombosis, more than 1 fetal loss, FGR	Italy	Case control	S	1/263
		TM, c.1208G>A (EGF region)	Late pregnancy loss (>20 wk)	Italy	Case control	S	0/263
		TM, c. 282C>G (Lectin-like region)	Late pregnancy loss (>20 wk), Plt thrombosis, FGR	Italy	Case control	S	0/263
		TM, c.1502C>T (serine/threonine region)	Late pregnancy loss (>20 wk), more than 1 fetal loss	Italy	Case control	S	2/263
1999	Nakabayashi et al.[52]	Val455- TM allele (e6 domain EGF)	Severe PE (early onset)	Japan	Case control	N	37.50%
		Ala455- TM allele (e6 domain EGF)	Severe PE (early onset)	Japan	Case control	N	62.50%
2012	Said et al.[50]	TM, c.C1418T homozygous (EGF region)	Severe PE, FGR, placental abruption, IUFD	Australia	Case control	N	4/115
		TM, c.C1418T heterozygous (EGF region)	Severe PE, FGR, placental abruption, IUFD	Australia	Case control	N	35/115
2002	Borg et al.[53]	Val 455- TM allele (e6 domain EGF region)	PE	Australia/New Zealand	Cohort	N	23.20%
		Ala455- TM allele (e6 domain EGF region)	PE	Australia/New Zealand	Cohort	N	76.80%
2012	Guerra et al.[55]	TM, c.C1418T (EGF region)	Recurrent pregnancy loss	Brazil	Case control	N	60/264

Abbreviations: N, not significant; S, significant.

4.1 | Early pregnancy loss

Maternal thrombophilia is one of the acknowledged risk factors of early pregnancy loss. Placentation relies on sufficient trophoblast invasion into the endometrium under suppressed local inner immunity and vascular formation with anticoagulation properties. TM is involved in the regulation of coagulation and inflammatory reactions; thus, it is reasonable to consider TM as a pivotal player in the process of placentation. The association between TM deficiency and adverse pregnancy outcomes has been assessed based on the hypothesis that altered TM functions may impair placentation by inducing prothrombotic status. Although not a major cause, several TM mutations may be associated with recurrent miscarriage.[48,68] Franchi et al. reported that mothers with TM mutations are at an increased risk of unexplained fetal death in late pregnancy.[49] Blood serum collected from patients experiencing pregnancy loss shows high sTM levels compared with normal pregnancy at the same gestational stage.[69] Because the study of women diagnosed with recurrent pregnancy loss (RPL) showed no significant elevation of sTM levels in their nonpregnant status,[70] the enhancement of sTM is likely to occur in response to pregnancy. RPL patients lack TM expression in the endometrium,[57] indicating the relevance of TM in the process of decidualization. Indeed, placental tissues collected upon miscarriage show diminished TM expressions that supports this speculation.[71] TM may be involved in the initial interaction of the trophoblast and endometrium, which determines the receptivity of embryo implantation and subsequent placentation.

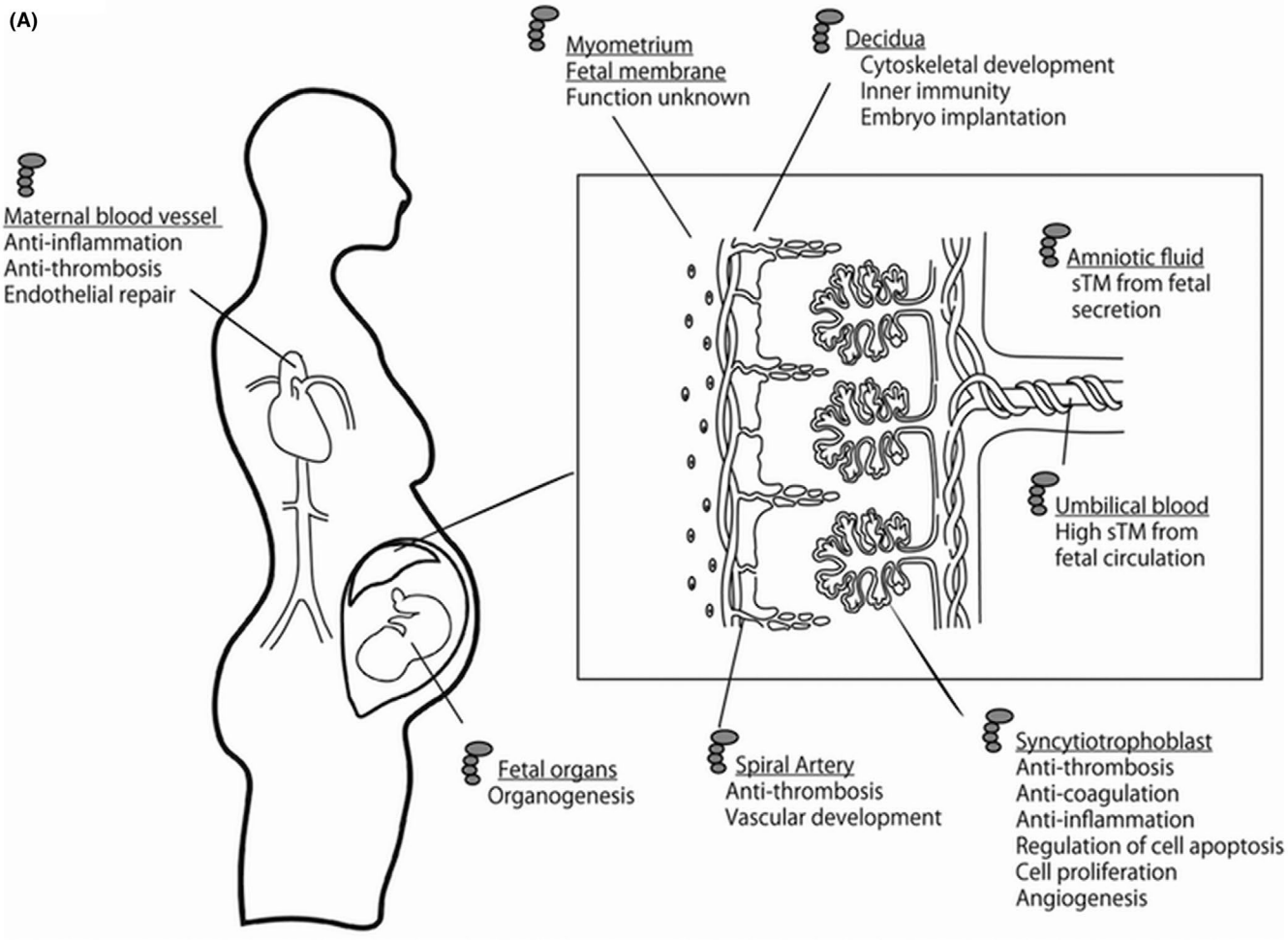
4.2 | FGR and intrauterine fetal death

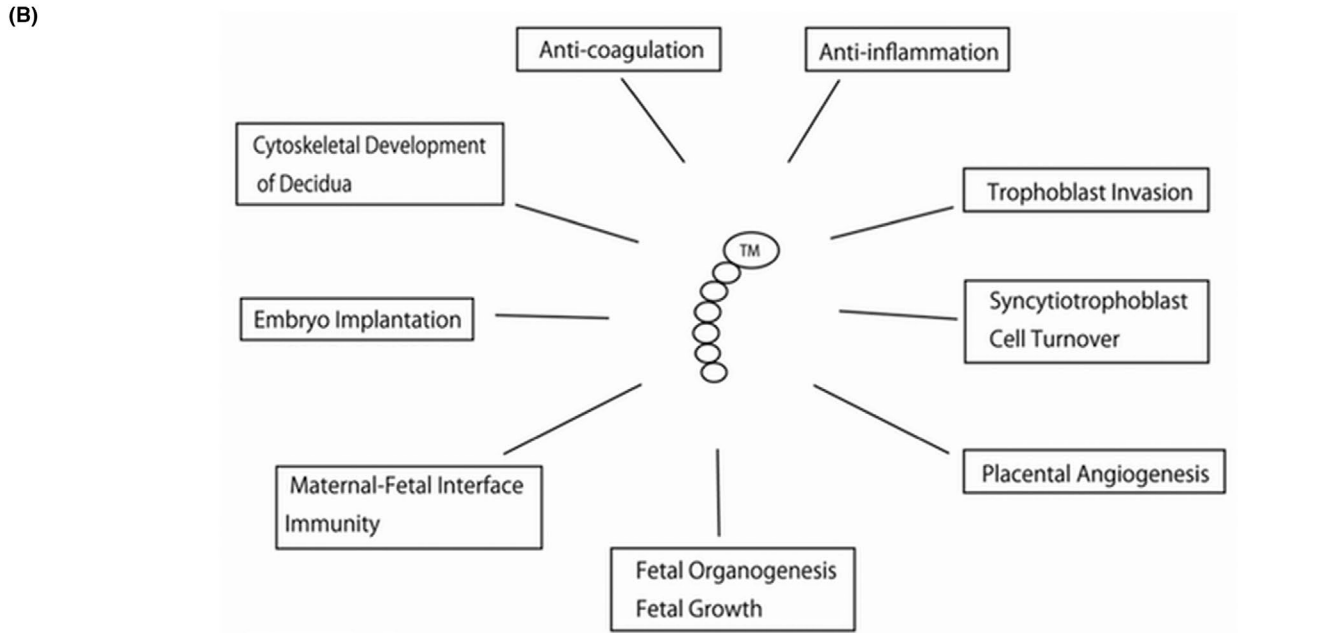
Insufficient blood supply resulting from poor placentation may result in FGR and even intrauterine fetal death. Although the FVL mutation or prothrombin mutation is known to be involved in the occurrence of FGR,[47,72] the relevance of TM mutations remains controversial. TM gene mutations in the promoter region (TM, c.201-G>A) and lectin-like region (TM, c. 282C>G) were specifically found in patients with severe FGR, which could be a potential risk factor for placental dysfunction.[49] TM protein and mRNA expression in syncytiotrophoblast were comparable between FGR and normal pregnancy.[61,73] However, TM protein levels in the placental villous capillary endothelium were increased in the FGR group.[61] Serum sTM levels negatively correlate with fetal weights in mothers with PE and FGR,[74] which may reflect the lack of functional cell-surface TM under such conditions. Although it has long been believed that TM at the maternal-fetal interface prevents placental thrombosis and supports fetal growth, the pathological severity of placental thromboembolism does not correlate with the occurrence of adverse fetal outcomes.[75] Indeed, the extent of placental thrombosis does not correlate with placental TM expression levels in TM-gene-deficient mice,[32] suggesting that the anti-thrombotic function of TM may not be responsible for FGR. Recently, TM is reported to play a role in hypoxic stress reaction and subsequent angiogenesis.[46,73] Increased TM in the capillary endothelium in FGR placentas could be a compensatory reaction to rescue the placenta from hypoxia and insufficient vascular development. Placental TM could play a role in the compensatory process secondary to unfavorable conditions thereby supporting fetal growth.

4.3 | Preeclampsia

Numerous studies have supported the possible involvement of TM in the pathophysiology of PE. Several TM gene polymorphisms were shown to be related to the development of PE.[52] Circulating sTM levels are elevated in mothers with PE, independent of insufficient clearance caused by renal or hepatic dysfunction.[63–66] Mothers with chronic hypertension showed lower serum sTM levels than those with PE, implying that sTM does not simply reflect endothelial damage from hypertension.[76] Maternal sTM levels correlate with the clinical severity of PE[77] and are found to be higher in early-onset cases than in late-onset cases.[78,79] Preincubation of plasma obtained from mothers with PE did not enhance TM shedding in cultured umbilical endothelial cells. However, when neutrophils were cocultured, sTM increased in the media, suggesting that PE patient plasma contains neutrophil activation factors and cleaves endothelial TM.[66] The source of elevated sTM could be either the blood vessel endothelium or placental trophoblasts. Given that maternal sTM levels are elevated during pregnancy and that placental defects are the underlying cause of PE, it would be reasonable to assume that the majority of sTM observed in PE patients originate from the placenta. Therefore, sTM levels in PE may reflect the extent of placental damage, which may be relevant to the severity of the disease. These studies indicate that the elevation of maternal sTM levels could be considered as a biological marker associated with the pathology of PE. Immunohistochemical staining showed a significant loss of TM expression in syncytiotrophoblast of the placenta from PE patients, accompanied by an increase in antiangiogenic factor sFlt-1 mRNA expression.[73] TM mRNA positively correlate with placental MMP 2 and

FIGURE 2 Schematic of thrombomodulin (TM) functions during pregnancy: TM is expressed on various gestational organs with each of them playing a different role (A and B). TM expressed on the endothelium of maternal systemic blood vessels and placental spiral arteries regulate coagulation, inflammation, endothelial repair function, and vascular development that assures placental circulation. TM expressed on the endometrium (decidua) is involved in cytoskeletal development that determines inner immunity upon embryonic implantation. On the syncytiotrophoblast cells, TM serves as a bridging player between coagulation and inflammatory pathways, as well as the regulation of local angiogenesis, cell apoptosis and proliferation responsible for placental homeostasis. Soluble-TM (sTM) increase in maternal serum during pregnancy. Because TM is essential in fetal organogenesis, a high level of sTM is detected in fetal serum and in amniotic fluid





9 expression, which may regulate the invasion of trophoblasts.[73] Placental TM expression in oocyte donation (OD) pregnancies has been recently assessed.[80] Immunohistochemical studies showed a significant reduction in placental TM in mothers conceived by OD, regardless of whether they developed PE. TM mRNA expression in the placenta was even more diminished in OD mothers

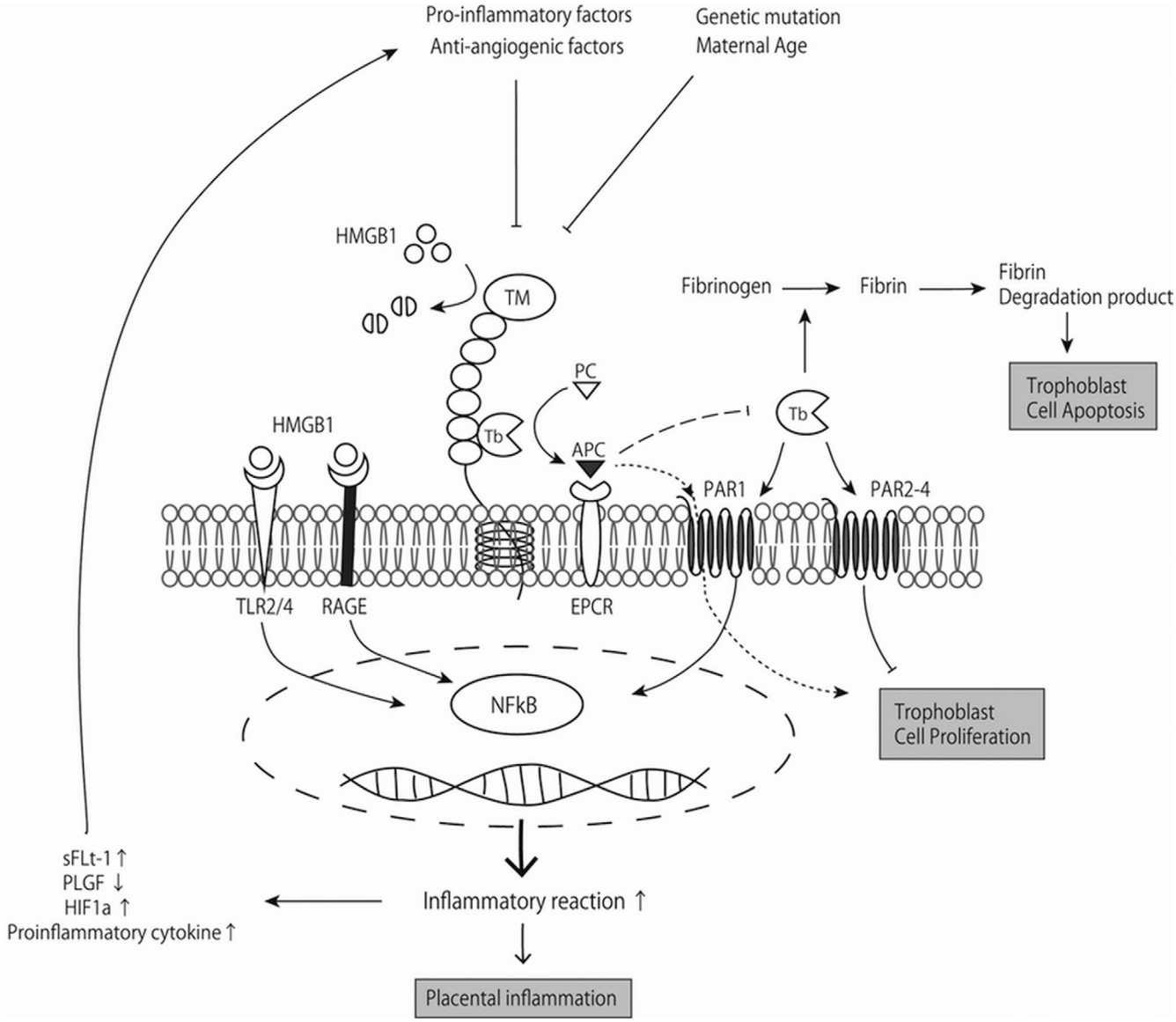


FIGURE 3 Molecular function of thrombomodulin (TM) in the placenta. Placental TM expression is regulated by angiogenic or genetic factors and possibly by maternal age. Thrombin (Tb) binds to TM forming a Tb-TM complex that enhances protein C (PC) activity. Activated PC (APC) further prevents Tb from converting fibrinogen to fibrin and subsequently decreasing fibrin degradation product, which is a causal factor of trophoblast cell apoptosis. APC also regulates G protein–coupled protease-activated receptor (PAR) 1–4 activities that promotes trophoblast cell proliferation. Extracellular high mobility group box1(HMGB1) activates Toll-like receptor (TLR) 2, TLR4, and receptor for advanced glycation end products (RAGE) that in turn enhance NF-κB mediated inflammatory signaling. This reaction caused by HMGB1 subsequently upregulates local HIF1a activity, antiangiogenic factor sFlt-1, and down-regulates proangiogenic factor; placenta growth factor (PlGF) in the placental tissue. TM inactivates this series of inflammation and antiangiogenic pathways by cleaving HMGB1

who had developed PE compared with uncomplicated OD mothers. Interestingly, these mRNA levels were inversely correlated with maternal age.[80] OD pregnancy is immunologically challenging because of the allogenic fetus. Considering the function of TM in the regulation of the immune system, the loss of placental TM in OD mothers could be either a cause of reduced immunological tolerance or the consequence of an overactivated immune system resulting in the cleavage of TM.

Most recently, a study focused on placental inflammation induced by extracellular vesicles (EV) has further elucidated the anti-inflammatory role of placental TM in PE pathogenesis.[81] EVs are proinflammatory substances that can cause PE by enhancing the activities of platelets, inflammasomes, and IL-1β. EV-dependent IL-1β directly suppresses TM expression and proliferation in trophoblast cells. EV infusion induced PE-like symptoms in pregnant mice, presenting placental inflammation and FGR.[81] These EV-induced symptoms were reversed by soluble-TM treatment, genetic placental-TM restoration, or IL-1β receptor antagonist, which prevented TM shedding. This study indicates that EV induces placental damage via reduction of TM, which compromises trophoblast cell proliferation and placental development, leading to the development of PE. These findings add a new dimension to the pathological function of placental TM in inflammatory pregnancy complications.

The pathophysiology of PE is systemic endothelial damage from overly produced proinflammatory factors from the dysfunctional placenta. Characteristic features of PE include trophoblast cell apoptosis, suppressed placental angiogenesis, systemic endothelial dysfunction, and enhanced inflammatory responses from activated proinflammatory factors.[82] TM is involved in placental development and maintenance, by regulating trophoblast cell turnover and physiological stress coping mechanisms.[31,41,46] These roles of TM may be central to the homeostasis of placental function. Although it may not be a direct cause of the disease, the lack of TM function during pregnancy may contribute to the development of PE.

5 | CLINICAL APPLICATION OF RECOMBINANT TM IN PREGNANCY-RELATED DISEASE

The clinical application of recombinant human soluble TM (ART123; rTM) has further widened the knowledge of TM. In the following section, we review the therapeutic mechanism of rTM and discuss its potential therapeutic application in pregnancy complications (Table 3).

5.1 | rTM and its therapeutic potential for non-obstetric diseases

Recombinant TM is a clinical agent that comprises functionally active extracellular domains of human TM.[83] Although the structure is almost identical to the sTM cleaved from the cell surface, the biological function of sTM and therapeutic rTM can be very different. The cleavage site of cell surface TM may depend on the cause of the cell damage, thus sTM fractions could be functionally unequal. Furthermore, TM activity is easily altered by stress factors such as oxidation.[40] Because TM shedding mostly occurs upon cell damage from environmental stress, it is highly possible that sTM is functionally impaired compared with therapeutic rTM agents. Daily intravenous administration of rTM is recommended for DIC treatment. Because of its anti-inflammatory effect, the efficacy of rTM has been particularly verified in sepsis-related DIC. The meta-analysis of three randomized control trials including 838 patients and nine observational studies showed a decrease in mortality rate in rTM-treated sepsis-induced DIC patients.[84] The most recent multinational randomized control trial enrolled 800 patients with sepsis-associated coagulopathy (SCARLET study) and showed a 2.6% reduction in the absolute risk of mortality in the rTM-treated group; however, the therapeutic efficacy did not reach statistical significance.[85] Although multiple studies show favorable trends in patient outcomes, further studies are required. The efficacy of rTM has been recently investigated in inflammatory diseases besides sepsis. Clinical trials have shown that rTM increases mortality in patients with idiopathic pulmonary fibrosis (IPF).[86] Because IPF is a sterile inflammatory disease with pathological relevance to HMGB1 activity, the inhibition of HMGB1 is considered to be the underlying therapeutic mechanism. Solulin (ZK158 266) is another recombinant TM agent with mutation in its N-terminal and C-terminal, which makes the agent resistant to proteolysis and oxidation. Because the benefit of Solulin is focused on its stability as an anticoagulant, the binding ability to the interfering protein is disrupted.[87] The N-terminal of TM is responsible for anti-inflammatory function. Thus, Solulin is likely to have less anti-inflammatory effect compared with native TM. In comparison, rTM (ART123) is a complete active agent of extracellular native TM. Considering the study results pointing out the relevance of its anti-inflammatory function during pregnancy, rTM could be the most ideal therapeutic agent for pregnancy complications.

5.2 | rTM as an anticoagulant agent for postpartum hemorrhage

Patients with DIC induced by obstetric complications are good candidates for rTM treatment. Several studies have reported the efficacy of rTM on clinical and laboratory improvement in DIC patients with obstetric complications such as postpartum hemorrhage, amniotic fluid embolism, and PE. A retrospective cohort study of obstetric DIC patients showed that postpartum rTM treatment significantly improved coagulant markers and reduced the amount of blood transfusion.[88–90] Obstetric DIC is not simply induced by hemorrhage upon delivery, but results from systemic inflammation caused by the entry of fetal components into the maternal circulation serving as a proinflammatory mediator. Amniotic fluid embolism is a typical condition caused by fetal substances in the maternal blood flow; however, contamination of fetal components is also found in postpartum hemorrhage and placental abruption.[91,92] Because these conditions frequently develop severe coagulopathy, the proinflammatory activity induced by fetal components is considered to play a role in the pathogenesis of obstetric DIC. Among various inflammatory signaling pathways, amniotic fluid is known to activate the complement system and its subsequent immune responses.[92,93] Because C3 and C5 are anti-inflammatory targets of rTM, the inhibitory effect of complement systems might explain its particular efficacy on obstetric DIC.

5.3 | rTM as an anti-inflammatory agent for PE

Because rTM fully retains the function of native cellular TM, it is also known for its active anti-inflammatory effect.[94] The unique inflammatory suppression properties of rTM, has extended its therapeutic application to sterile inflammatory diseases. In animal studies, promising results of rTM have been reported in inflammatory bowel disease and IPF.[86,95] The underlying etiology of PE is systemic inflammation because of insufficient placentation. Lack of placental TM expression were noted in mothers with PE. Hence, theoretically, rTM could replenish the function of the reduced placental TM function, thereby serving as a potential therapeutic for PE. Based on this perspective, several studies have assessed the efficacy of

TABLE 3 Studies assessing the therapeutic efficacy of rTM (ART123) in pregnancy complications

Year	Author	Assessed complications	Study model	Summary
2014	Shin et al.[98]	PE	Rat	rTM administration improved FBW, placental blood perfusion and fetal brain oxygenation in L-NAME +LPS induced PE rat.
2018	Sano et al.[99]	Recurrent miscarriage/ FGR	Mice	rTM decreased fetal resorption and placental fibrinogen deposition in CBA/J×DBA/2 recurrent miscarriage mice model. rTM increased FBW and placental VEGF expression.
2021	Oda et al.[96]	PE	Mice/human trophoblast cells	rTM attenuated HTN, proteinuria, FGR, and placental vasculature in Ang II-induced PE mice. sFlt−1, IL−6, and TNF-α were suppressed by rTM via HMBG1 in human trophoblast cells.
2021	Oda et al.[46]	Fetal growth	Mice/human trophoblast cells	rTM increased FBW and F/P-weight ratios in pregnant mice. rTM downregulates HIF1α and induces PlGF via HMGB1 in human trophoblast cells.
2013	Sugawara et al.[88]	PPH+DIC	Patient study	Single-center retrospective review of 36 patients. D-dimer levels and the frequency of bleeding episodes decreased in patients treated with rTM.
2015	Yoshihara et al.[89]	PPH+DIC	Patient study	Single-center retrospective cohort of 66 patients. Platelet transfusion volume was lower in the rTM treated patients.
2017	Kobayashi et al.[90]	PPH+DIC	Patient study	Postmarketing surveillance of 117 patients showed significant improvement in fibrinogen/fibrin degradation products, D-dimer, fibrinogen, PT/aPTT time in rTM treated patients.

Abbreviations: AngII, angiotensin2; DIC, disseminated intravascular coagulation; F/P weight ratio, fetal/placental weight ratio; FBW, fetal body weight; FGR, fetal growth restriction; HGBM1, high mobility group box1; PE, preeclampsia; PlGF, placental growth factor; PPH, postpartum hemorrhage; rTM, recombinant thrombomodulin (ART123).

rTM in animal models of PE. In our previous report, rTM administration to angiotensin 2-induced PE mouse significantly improved PE symptoms, including hypertension, proteinuria, FGR, and placental defects.[96] We further discovered that extracellular HMGB1 could induce trophoblast cell damage via NF-κB activation, resulting in excessive antiangiogenic factor sFlt-1 and proinflammatory cytokine production from the placenta.[96] Therefore, the underlying therapeutic mechanism of rTM could be the inhibition of HMGB1 activity, which subsequently protects the placenta from inflammatory damage. Recent studies have revealed the involvement of HMGB1 in various pregnancy-related pathologies such as FGR and preterm labor.[97] Given the findings that these complications underlie inflammation resulting from HMGB1 activity, rTM could be a potential therapeutic for these maternal complications, serving as an HMGB1 inhibitor.

Shin et al. reported the efficacy of rTM as an improvement of placental blood perfusion on a PE rat model, which further supports the efficacy of rTM on PE.[98] Another study using CBA/J × DBA/2 mice[99] has concluded that rTM may reduce fetal resorption in RPL patients. Although its safety is clinically proven in human, the use of rTM during pregnancy is contraindicated because of the theoretical concern of adverse events. Indeed, animal studies have reported systemic hemorrhage in dams treated with extremely high-dose rTM. However, rTM is a large molecule > 64 kDa in size, making it highly unlikely to cross the placental barrier and affect the fetus. Given the results of the listed studies, it is quite possible that the appropriate dose of rTM could be a safe therapeutic approach for PE and other pregnancy complications. Last, it is important to note that each animal model of pathological pregnancy has its limitations. The therapeutic effect of rTM should be verified in multiple animal models for solid evidence.

6 | CONCLUSION

Thrombomodulin is a multifaceted transmembrane protein that facilitates various biochemical signaling and physiological pathways. In this review, we have clarified its organ-protective functions that could be central to placental homeostasis throughout pregnancy. TM mediates apoptosis and proliferation of trophoblast cells. It is also involved in the crosstalk of coagulation, inflammation, and angiogenesis, which are the compensating mechanisms for environmental

stress that directly affect placental function. Because accumulating evidence suggests the possible relevance of placental-TM deficiency in adverse pregnancy outcomes, a number of questions could be raised throughout this review. What exactly is regulating the placental-TM expression and when does it start? The lack of placental-TM could be either genetic or acquired, which provokes our next question: is diminished placental-TM the cause or the consequence of pregnancy complications? Most importantly, rTM shows promising potential as a therapeutic for pregnancy complication in animal studies. Considering the successful results of clinical trials in other inflammatory disease, there is no doubt that rTM is an optimal therapeutic target for PE, FGR, and RPL. TM could drastically change the therapeutic approach to pregnancy complications that may significantly improve maternal and fetal outcomes.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Hiroko Oda wrote the manuscript. Takeshi Nagamatsu and Yutaka Osuga contributed in supervising the whole project.

INFORMED CONSENT

All the authors have read and approved the submission. Full informed consent was obtained for this article from all the co-authors.

ORCID

Hiroko Oda https://orcid.org/0000-0002-8268-1529
Takeshi Nagamatsu https://orcid.org/0000-0003-1480-5785

REFERENCES

1. Esmon CT, Owen WG. Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. *Proc Natl Acad Sci USA*. 1981;78:2249-2252.
2. Maruyama I, Bell CE, Majerus PW. Thrombomodulin is found on endothelium of arteries, veins, capillaries, and lymphatics, and on syncytiotrophoblast of human placenta. *J Cell Biol*. 1985;101(2):363-371.
3. Dittman WA, Majerus PW. Structure and function of thrombomodulin: a natural anticoagulant. *Blood*. 1990;75:329-336.
4. Abeyama K, Stern DM, Ito Y, et al. The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel anti-inflammatory mechanism. *J Clin Invest*. 2005;115(5):1267-1274.
5. Lin WL, Chen CC, Shi GY, Ma CY, Chang C F, Wu H L. Monocytic thrombomodulin promotes cell adhesion through interacting with its ligand, Lewis(y). *Immunol Cell Biol*. 2017;95:372-379.
6. Zushi M, Gomi K, Yamamoto S, Maruyama I, Hayashi T, Suzuki K. The last three consecutive epidermal growth factor-like structures of human thrombomodulin comprise the minimum functional domain for protein C-activating cofactor activity and anticoagulant activity. *J Biol Chem*. 1989;264:10351-10353.
7. Shi CS, Shi GY, Chang YS, et al. Evidence of human thrombomodulin domain as a novel angiogenic factor. *Circulation*. 2005;111:1627-1636.
8. Boehme MW, Deng Y, Raeth U, et al. Release of thrombomodulin from endothelial cells by concerted action of TNF-alpha and neutrophils: in vivo and in vitro studies. *Immunology*. 1996;87:134-140.
9. Hsu YY, Shi GY, Kuo CH, et al. Thrombomodulin is an ezrin-interacting protein that controls epithelial morphology and promotes collective cell migration. *FASEB J*. 2012;26:3440-3452.
10. Ishii H, Majerus PW. Thrombomodulin is present in human plasma and urine. *J Clin Invest*. 1985;76:2178-2181.
11. Takano S, Kimura S, Ohdama S, Aoki N. Plasma thrombomodulin in health and diseases. *Blood*. 1990;76:2024-2029.
12. Gando S, Kameue T, Nanzaki S, Nakanishi Y. Cytokines, soluble thrombomodulin and disseminated intravascular coagulation in patients with systemic inflammatory response syndrome. *Thromb Res*. 1995;80(6):519-526.
13. Isermann B, Hendrickson SB, Zogg M, et al. Endothelium-specific loss of murine thrombomodulin disrupts the protein C anticoagulant pathway and causes juvenile-onset thrombosis. *J Clin Invest*. 2001;108:537-546.
14. Esmon CT, Esmon NL, Harris KW. Complex formation between thrombin and thrombomodulin inhibits both thrombin-catalyzed fibrin formation and factor V activation. *J Biol Chem*. 1982;257:7944-7947.
15. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature*. 1994;369:64-67.
16. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. *J Biol Chem*. 1996;271(28):16603-16608.
17. Bouma BN, Meijers JC. Thrombin-activatable fibrinolysis inhibitor (TAFI, plasma procarboxypeptidase B, procarboxypeptidase R, procarboxypeptidase U). *J Thromb Haemost*. 2003;1(7):1566-1574.
18. Wang W, Nagashima M, Schneider M, Morser J, Nesheim M. Elements of the primary structure of thrombomodulin required for efficient thrombin-activable fibrinolysis inhibitor activation. *J Biol Chem*. 2000;275:22942-22947.
19. Weiler H, Lindner V, Kerlin B, et al. Characterization of a mouse model for thrombomodulin deficiency. *Arterioscler Thromb Vasc Biol*. 2001;21:1531-1537.
20. Joyce DE, Gelbert L, Ciaccia A, DeHoff B, Grinnell BW. Gene expression profile of antithrombotic protein c defines new mechanisms modulating inflammation and apoptosis. *J Biol Chem*. 2001;276:11199-11203.
21. Bae JS, Rezaie AR. Thrombin inhibits nuclear factor kappaB and RhoA pathways in cytokine-stimulated vascular endothelial cells when EPCR is occupied by protein C. *Thromb Haemost*. 2009;101:513-520.
22. Bae JS, Yang L, Manithody C, Rezaie AR. The ligand occupancy of endothelial protein C receptor switches the protease-activated receptor 1-dependent signaling specificity of thrombin from a permeability-enhancing to a barrier-protective response in endothelial cells. *Blood*. 2007;110(12):3909-3916.
23. Healy LD, Puy C, Fernandez JA, et al. Activated protein C inhibits neutrophil extracellular trap formation in vitro and activation in vivo. *J Biol Chem*. 2017;292:8616-8629.
24. Campbell WD, Lazoura E, Okada N, Okada H. Inactivation of C3a and C5a octapeptides by carboxypeptidase R and carboxypeptidase N. *Microbiol Immunol*. 2002;46(2):131-134.
25. Delvaeye M, Noris M, De Vriese A, et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med*. 2009;361:345-357.
26. Van de Wouwer M, Plaisance S, De Vriese A, et al. The lectin-like domain of thrombomodulin interferes with complement activation and protects against arthritis. *J Thromb Haemost*. 2006;4:1813-1824.
27. Wang H, Vinnikov I, Shahzad K, et al. The lectin-like domain of thrombomodulin ameliorates diabetic glomerulopathy via complement inhibition. *Thromb Haemost*. 2012;108:1141-1153.
28. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol*. 2005;5(4):331-342.

29. Ito T, Kawahara K, Okamoto K, et al. Proteolytic cleavage of high mobility group box 1 protein by thrombin-thrombomodulin complexes. *Arterioscler Thromb Vasc Biol*. 2008;28(10):1825-1830.
30. Conway EM, Van de Wouwer M, Pollefeyt S, et al. The lectin-like domain of thrombomodulin confers protection from neutrophil-mediated tissue damage by suppressing adhesion molecule expression via nuclear factor kappaB and mitogen-activated protein kinase pathways. *J Exp Med*. 2002;196:565-577.
31. Sood R, Weiler H. Embryogenesis and gene targeting of coagulation factors in mice. *Best Pract Res Clin Haematol*. 2003;16:169-181.
32. Isermann B, Hendrickson SB, Hutley K, Wing M, Weiler H. Tissue-restricted expression of thrombomodulin in the placenta rescues thrombomodulin-deficient mice from early lethality and reveals a secondary developmental block. *Development*. 2001;128:827-838.
33. Conway EM, Pollefeyt S, Cornelissen J, et al. Structure-function analyses of thrombomodulin by gene-targeting in mice: the cytoplasmic domain is not required for normal fetal development. *Blood*. 1999;93:3442-3450.
34. Imada S, Yamaguchi H, Nagumo M, Katayanagi S, Iwasaki H, Imada M. Identification of fetomodulin, a surface marker protein of fetal development, as thrombomodulin by gene cloning and functional assays. *Dev Biol*. 1990;140(1):113-122.
35. Ford VA, Wilkinson JE, Kennel SJ. Thrombomodulin distribution during murine development. *Rouxs Arch Dev Biol*. 1993;202:364-370.
36. Healy AM, Rayburn HB, Rosenberg RD, Weiler H. Absence of the blood-clotting regulator thrombomodulin causes embryonic lethality in mice before development of a functional cardiovascular system. *Proc Natl Acad Sci USA*. 1995;92:850-854.
37. van Mens TE, Liang HH, Basu S, et al. Variable phenotypic penetrance of thrombosis in adult mice after tissue-selective and temporally controlled Thbd gene inactivation. *Blood Adv*. 2017;1:1148-1158.
38. Sood R, Zogg M, Westrick RJ, et al. Fetal gene defects precipitate platelet-mediated pregnancy failure in factor V Leiden mothers. *J Exp Med*. 2007;204:1049-1056.
39. Weiler-Guettler H, Christie PD, Beeler DL, et al. A targeted point mutation in thrombomodulin generates viable mice with a prethrombotic state. *J Clin Invest*. 1998;101:1983-1991.
40. Glaser CB, Morser J, Clarke JH, et al. Oxidation of a specific methionine in thrombomodulin by activated neutrophil products blocks cofactor activity. A potential rapid mechanism for modulation of coagulation. *J Clin Invest*. 1992;90:2565-2573.
41. Isermann B, Sood R, Pawlinski R, et al. The thrombomodulin-protein C system is essential for the maintenance of pregnancy. *Nat Med*. 2003;9:331-337.
42. Weiler H, Isermann BH. Thrombomodulin. *J Thromb Haemost*. 2003;1:1515-1524.
43. Gu JM, Crawley JT, Ferrell G, et al. Disruption of the endothelial cell protein C receptor gene in mice causes placental thrombosis and early embryonic lethality. *J Biol Chem*. 2002;277:43335-43343.
44. Sood R, Sholl L, Isermann B, Zogg M, Coughlin SR, Weiler H. Maternal Par4 and platelets contribute to defective placenta formation in mouse embryos lacking thrombomodulin. *Blood*. 2008;112(3):585-591.
45. Vogel S, Bodenstein R, Chen Q, et al. Platelet-derived HMGB1 is a critical mediator of thrombosis. *J Clin Invest*. 2015;125(12):4638-4654.
46. Oda H, Nagamatsu T, Cabral H, et al. Thrombomodulin promotes placental function by up-regulating placental growth factor via inhibition of high-mobility-group box 1 and hypoxia-inducible factor 1alpha. *Placenta*. 2021;111:1-9.
47. Kupferminc MJ, Eldor A, Steinman N, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med*. 1999;340:9-13.
48. Cao Y, Zhang Z, Xu J, et al. The association of idiopathic recurrent pregnancy loss with polymorphisms in hemostasis-related genes. *Gene*. 2013;530:248-252.
49. Franchi F, Biguzzi E, Cetin I, et al. Mutations in the thrombomodulin and endothelial protein C receptor genes in women with late fetal loss. *Br J Haematol*. 2001;114:641-646.
50. Said JM, Higgins JR, Moses EK, Walker SP, Monagle PT, Brennecke SP. Inherited thrombophilias and adverse pregnancy outcomes: a case-control study in an Australian population. *Acta Obstet Gynecol Scand*. 2012;91:250-255.
51. Wiwanitkit V. Correlation between thrombomodulin and severe pre-eclampsia: a summary. *Clin Appl Thromb Hemost*. 2008;14:99-101.
52. Nakabayashi M, Yamamoto S, Suzuki K. Analysis of thrombomodulin gene polymorphism in women with severe early-onset pre-eclampsia. *Semin Thromb Hemost*. 1999;25:473-479.
53. Borg AJ, Higgins JR, Brennecke SP, Moses EK. Thrombomodulin Ala455Val dimorphism is not associated with pre-eclampsia in Australian and New Zealand women. *Gynecol Obstet Invest*. 2002;54(1):43-45.
54. Hira B, Pegoraro RJ, Rom L, Moodley J. Absence of factor V Leiden, thrombomodulin and prothrombin gene variants in Black South African women with pre-eclampsia and eclampsia. *BJOG*. 2003;110(3):327-328.
55. Guerra-Shinohara EM, Bertinato JF, Tosin Bueno C, et al. Polymorphisms in antithrombin and in tissue factor pathway inhibitor genes are associated with recurrent pregnancy loss. *Thromb Haemost*. 2012;108:693-700.
56. Buurma AJ, Penning ME, Prins F, et al. Preeclampsia is associated with the presence of transcriptionally active placental fragments in the maternal lung. *Hypertension*. 2013;62:608-613.
57. D'Ippolito S, Di Nicuolo F, Papi M, et al. Expression of pinopodes in the endometrium from recurrent pregnancy loss women. Role of thrombomodulin and Ezrin. *J Clin Med*. 2020;9:2634.
58. Salem HH, Maruyama I, Ishii H, Majerus PW. Isolation and characterization of thrombomodulin from human placenta. *J Biol Chem*. 1984;259:12246-12251.
59. Fazel A, Vincenot A, Malassine A, et al. Increase in expression and activity of thrombomodulin in term human syncytiotrophoblast microvilli. *Placenta*. 1998;19:261-268.
60. Uszynski M, Sztenc S, Zekanowska E, Uszynski W. Thrombomodulin in human gestational tissues: placenta, fetal membranes and myometrium. *Adv Med Sci*. 2006;51:312-315.
61. Wienhard J, Bielska B, Munstedt K, Lang U, Zygmunt M. Increased endothelial thrombomodulin (TM) expression in pregnancies complicated by IUGR. *J Perinat Med*. 2002;30:322-328.
62. de Moerloose P, Mermillod N, Amiral J, Reber G. Thrombomodulin levels during normal pregnancy, at delivery and in the postpartum: comparison with tissue-type plasminogen activator and plasminogen activator inhibitor-1. *Thromb Haemost*. 1998;79(03):554-556.
63. Minakami H, Takahashi T, Izumi A, Tamada T. Increased levels of plasma thrombomodulin in preeclampsia. *Gynecol Obstet Invest*. 1993;36:208-210.
64. Rousseau A, Favier R, Van Dreden P. Elevated circulating soluble thrombomodulin activity, tissue factor activity and circulating procoagulant phospholipids: new and useful markers for pre-eclampsia? *Eur J Obstet Gynecol Reprod Biol*. 2009;146:46-49.
65. Dusse L, Godoi L, Kazmi RS, et al. Sources of thrombomodulin in pre-eclampsia: renal dysfunction or endothelial damage? *Semin Thromb Hemost*. 2011;37:153-7.
66. Kobayashi H, Sadakata H, Suzuki K, She MY, Shibata S, Terao T. Thrombomodulin release from umbilical endothelial cells initiated by preeclampsia plasma-induced neutrophil activation. *Obstet Gynecol*. 1998;92:425-430.
67. Uszynski M, Uszynski W, Zekanowska E, Kuczynski J, Szymanski M. A comparative study of the protein C system in mother's blood, cord blood and amniotic fluid. *Folia Histochem Cytobiol*. 2010;48:262-266.
68. Kaare M, Ulander VM, Painter JN, Ahvenainen T, Kaaja R, Aittomaki K. Variations in the thrombomodulin and endothelial protein C

receptor genes in couples with recurrent miscarriage. *Hum Reprod*. 2007;22:864-868.
69. Van Dreden P, Woodhams B, Rousseau A, Favier M, Favier R. Comparative evaluation of tissue factor and thrombomodulin activity changes during normal and idiopathic early and late foetal loss: the cause of hypercoagulability? *Thromb Res*. 2012;129:787-792.
70. de Larranaga GF, Remondino G, Alonso BS, Voto L. Soluble thrombomodulin levels among women with a history of recurrent pregnancy loss, with or without antiphospholipid antibodies. *Blood Coagul Fibrinolysis*. 2005;16(1):31-35.
71. Stortoni P, Cecati M, Giannubilo SR, et al. Placental thrombomodulin expression in recurrent miscarriage. *Reprod Biol Endocrinol*. 2010;8:1.
72. Kupferminc MJ, Many A, Bar-Am A, Lessing JB, Ascher-Landsberg J. Mid-trimester severe intrauterine growth restriction is associated with a high prevalence of thrombophilia. *BJOG*. 2002;109:1373-1376.
73. Turner RJ, Bloemenkamp KW, Bruijn JA, Baelde HJ. Loss of thrombomodulin in placental dysfunction in preeclampsia. *Arterioscler Thromb Vasc Biol*. 2016;36:728-735.
74. Varol FG, Ozgen L, Sayin NC, Demir M. Correlation between maternal plasma thrombomodulin and infant birth weight in hypertensive disorders of pregnancy. *Clin Appl Thromb Hemost*. 2009;15:166-170.
75. Salafia CM, Cowchock FS. Placental pathology and antiphospholipid antibodies: a descriptive study. *Am J Perinatol*. 1997;14:435-441.
76. Hsu CD, Copel JA, Hong SF, Chan DW. Thrombomodulin levels in preeclampsia, gestational hypertension, and chronic hypertension. *Obstet Gynecol*. 1995;86(6):897-899.
77. Dusse LM, Alpoim PN, Lwaleed BA, de Sousa LP, Carvalho M, Gomes KB. Is there a link between endothelial dysfunction, coagulation activation and nitric oxide synthesis in preeclampsia? *Clin Chim Acta*. 2013;415:226-229.
78. Heilmann L, Rath W, Pollow K. Hemostatic abnormalities in patients with severe preeclampsia. *Clin Appl Thromb Hemost*. 2007;13:285-291.
79. Alpoim PN, Perucci LO, Godoi LC, Goulart COL, Dusse LMS. Oxidative stress markers and thrombomodulin plasma levels in women with early and late severe preeclampsia. *Clin Chim Acta*. 2018;483:234-238.
80. Bos M, Baelde HJ, Bruijn JA, Bloemenkamp KW, van der Hoorn MP, Turner RJ. Loss of placental thrombomodulin in oocyte donation pregnancies. *Fertil Steril*. 2017;107:119-129.e5.
81. Kohli S, Singh KK, Gupta A, et al. Placental thromboinflammation impairs embryonic survival by reducing placental thrombomodulin expression. *Blood*. 2021;137:977-982.
82. Karumanchi SA, Granger JP. Preeclampsia and pregnancy-related hypertensive disorders. *Hypertension*. 2016;67:238-242.
83. Suzuki K, Kusumoto H, Deyashiki Y, et al. Structure and expression of human thrombomodulin, a thrombin receptor on endothelium acting as a cofactor for protein C activation. *EMBO J*. 1987;6(7):1891-1897.
84. Yamakawa K, Aihara M, Ogura H, Yuhara H, Hamasaki T, Shimazu T. Recombinant human soluble thrombomodulin in severe sepsis: a systematic review and meta-analysis. *J Thromb Haemost*. 2015;13:508-519.
85. Vincent J-L, Francois B, Zabolotskikh I, et al. Effect of a recombinant human soluble thrombomodulin on mortality in patients with sepsis-associated coagulopathy: the SCARLET randomized clinical trial. *JAMA*. 2019;321:1993-2002.
86. Sakamoto S, Shimizu H, Isshiki T, Sugino K, Kurosaki A, Homma S. Recombinant human soluble thrombomodulin for acute exacerbation of idiopathic pulmonary fibrosis: a historically controlled study. *Respir Investig*. 2018;56:136-143.
87. van Iersel T, Stroissnig H, Giesen P, Wemer J, Wilhelm-Ogunbiyi K. Phase I study of Solulin, a novel recombinant soluble human thrombomodulin analogue. *Thromb Haemost*. 2011;105(02):302-312.
88. Sugawara J, Suenaga K, Hoshiai T, et al. Efficacy of recombinant human soluble thrombomodulin in severe postpartum hemorrhage with disseminated intravascular coagulation. *Clin Appl Thromb Hemost*. 2013;19:557-561.
89. Yoshihara M, Uno K, Tano S, et al. The efficacy of recombinant human soluble thrombomodulin for obstetric disseminated intravascular coagulation: a retrospective study. *Crit Care*. 2015;19:369.
90. Kobayashi T, Kajiki M, Nihashi K, Honda G. Surveillance of the safety and efficacy of recombinant human soluble thrombomodulin in patients with obstetrical disseminated intravascular coagulation. *Thromb Res*. 2017;159:109-115.
91. Ohkawa R, Ohkawa K, Tosaka N, Ohhashi T, Hatano A, Ohkawa T. Complement system in abnormal pregnancy and chorionic neoplasia: immunotherapy and chemotherapy of chorionic neoplasia. *Adv Exp Med Biol*. 1984;176:111-125.
92. Kanayama N, Tamura N. Amniotic fluid embolism: pathophysiology and new strategies for management. *J Obstet Gynaecol Res*. 2014;40:1507-1517.
93. Benson MD, Kobayashi H, Silver RK, Oi H, Greenberger PA, Terao T. Immunologic studies in presumed amniotic fluid embolism. *Obstet Gynecol*. 2001;97:510-514.
94. Ito T, Thachil J, Asakura H, Levy JH, Iba T. Thrombomodulin in disseminated intravascular coagulation and other critical conditions-a multi-faceted anticoagulant protein with therapeutic potential. *Crit Care*. 2019;23:280.
95. Ueda T, Higashiyama M, Narimatsu K, et al. Recombinant thrombomodulin modulates murine colitis possibly via high-mobility group box 1 protein inhibition. *Digestion*. 2015;92:108-119.
96. Oda H, Nagamatsu T, Schust DJ, et al. Recombinant thrombomodulin attenuates preeclamptic symptoms by inhibiting high-mobility group box 1 in mice. *Endocrinology*. 2021;162(4):bqaa248.
97. Saito Reis CA, Padron JG, Norman Ing ND, Kendal-Wright CE. High-mobility group box 1 is a driver of inflammation throughout pregnancy. *Am J Reprod Immunol*. 2021;85:e13328.
98. Shin M, Hino H, Tamura M, et al. Thrombomodulin improves maternal and fetal conditions in an experimental pre-eclampsia rat model. *J Obstet Gynaecol Res*. 2014;40:1226-1234.
99. Sano T, Terai Y, Daimon A, et al. Recombinant human soluble thrombomodulin as an anticoagulation therapy improves recurrent miscarriage and fetal growth restriction due to placental insufficiency - The leading cause of preeclampsia. *Placenta*. 2018;65:1-6.

How to cite this article: Oda H, Nagamatsu T, Osuga Y. Thrombomodulin and pregnancy in the limelight: Insights into the therapeutic aspect of thrombomodulin in pregnancy complications. *J Thromb Haemost*. 2022;20:1040–1055. doi:10.1111/jth.15680