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Angiogenesis and Cancer

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Summary

Angiogenesis, the creation of neovasculatures from pre-existing ones, is required for various physiological processes. However, pathological angiogenesis is a hallmark of malignant tumors, metastasis and various ischemic as well as inflammatory disorders. Angiogenesis is regulated by the balance between proangiogenic factors and antiangiogenic factors, and concentrated effort in this area of research has led to the discovery of a growing number of angiogenesis-associated factors and the complex interactions among these factors. Understanding of the regulatory mechanisms of these factors in mediating the angiogenic process involved in tumor growth prompted the application of antiangiogenic factors on experimental tumor models with successful outcomes. Based on these experimental results, some antiangiogenic agents have been tested in clinical trials. In this review, the process and regulators of angiogenesis, the involvement of angiogenesis in cancer development and the application of antiangiogenic therapies on established tumors would be discussed. Among various antiangiogenic reagents, special emphasis will be given to antiangiogenic reagents derived from vascular basement membranes, a crucial regulator of angiogenesis, rather than a structural tissue component.

Key Words: Angiogenesis; endothelial cell; blood vessels; tumor; vascular basement membrane; endostatin; tumstatin; integrin; VEGF; angiopoietins.

1. Introduction

Angiogenesis, the formation of new capillaries from preexisting blood vessels, is required for several physiological processes as well as pathological conditions. In recent years, the involvement of angiogenesis in the development of malignant tumors and metastasis has been extensively studied. Therapeutic strategies to suppress angiogenesis has been examined on experimental tumor models with successful outcomes. Based on these experimental data, some antiangiogenic agents have been tested in clinical trials. In this review, the process and regulators of angiogenesis, the involvement of angiogenesis in cancer development, and the application of antiangiogenic therapies on established tumors will be discussed. Among various antiangiogenic reagents, special emphasis will be given to antiangiogenic reagents derived from vascular basement membranes.

2. The Process of Angiogenesis

Angiogenesis, the formation of new blood vessels from preexisting ones, is composed of several steps (*see* Fig. 1): (1) the degradation of vascular basement membrane matrix by protease, (2) migration and proliferation of endothelial cells into interstitium, (3) endothelial tube formation, (4) recruitment and attachment of mesenchymal cells to

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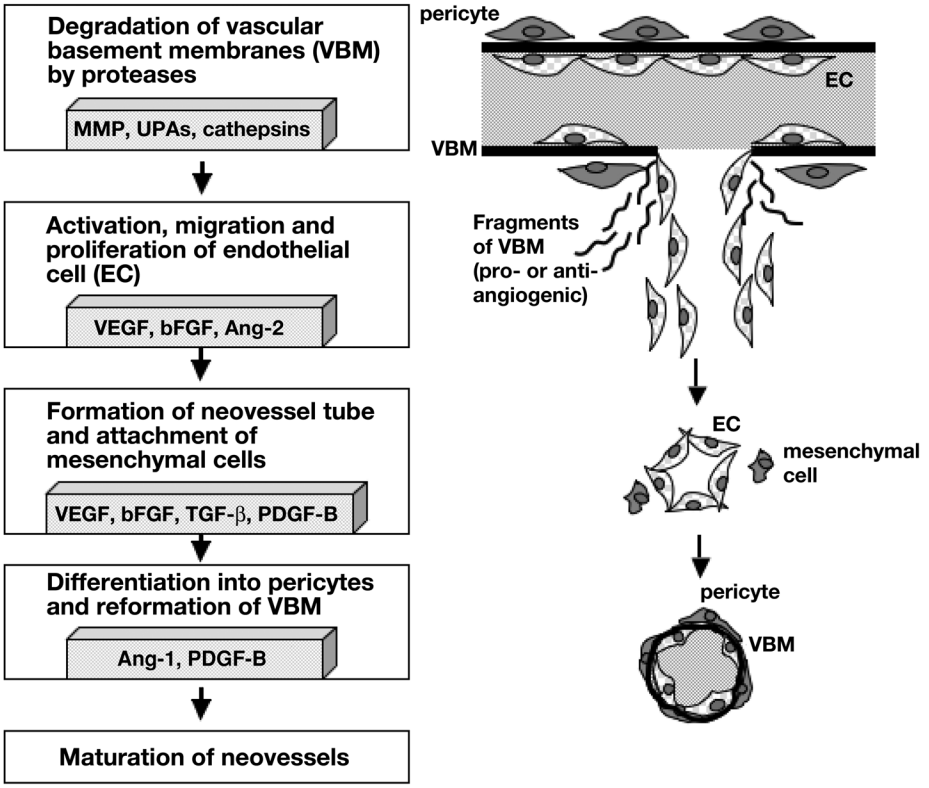

Fig. 1. The vascular microenvironment in the process of angiogenesis. Vascular basement membranes (VBM) are degraded by proteases such as MMPs in activated blood vessels. Proangiogenic growth factors (e.g.,VEGF, bFGF, PDGF) are released from VBM, produced by surrounding tumor cells, fibroblasts, and immune cells, and induce proliferation and migration of endothelial cells (EC). Fragments of VBM have pro- or antiangiogenic activities and regulate angiogenesis. Formation of EC tube with the attachment of pericytes leads to the maturation of neovessels with VBM.

the endothelial cell tube, and (5) maturation of blood vessels with the formation of vascular basement membrane *(1)*. In these angiogenic steps, several angiogenic factors and extracellular matrix (ECM) proteins are involved. Although small blood vessels consist only of endothelial cells, larger vessels are surrounded by mural cells such as pericytes and smooth muscle cells. In the quiescent stage, endothelial cells reside on vascular basement membranes mainly composed of type IV collagen, laminin, nidogen, and heparan sulfate proteoglycan (HSPG) *(2)*. Upon angiogenic stimuli, proteases represented by matrix metalloprotease (MMP) are activated and, in turn, degrade vascular basement membranes, thus leading to the migration of activated endothelial cells into interstitium. Proteinases also expose new cryptic epitopes and produce fragments of ECM proteins such as type IV collagen, potentially leading to the regulation of migratory capacity of endothelial cells as well as smooth muscle cells *(3)*. A provisional matrix of fibronectin, fibrin, and other components provides a supporting scaffold, guiding endothelial cells to their targets and thus facilitating neovessel formation. Proangiogenic growth factors, as well as these provisional matrix proteins, affect the

migration, proliferation, and tube formation of endothelial cells via specific cell surface receptors for those growth factors as well as integrin families. Integrin receptors are composed of heterodimers of α and β subunit and display a variety of biological functions upon binding to various matrix proteins and peptides, assisting vascular cells to build new vessels in coordination with their surrounding matrix environments *(4,5)*. Among various integrin families, the αvβ3 and αvβ5 integrins have been extensively investigated for possible involvement in angiogenic process, and pharmacological blockade of these integrins has been reported to suppress pathological angiogenesis *(6,7)*. However, genetic deletion studies of the αvβ3 and αvβ5 integrins suggest that integrins expressed on vascular endothelial cells inhibit angiogenesis by suppressing vascular endothelial growth factor (VEGF)-mediated endothelial cell survival, through transdominant effect on other proangiogenic integrins or by mediating the antiangiogenic activity of thrombospondin-1 and other angiogenesis inhibitors *(4,8,9)*. The possible positive or negative roles of integrins on angiogenesis depending on specific conditions thus require further clarification.

Recent advances in molecular biology have further revealed the involvement of antiangiogenic factors in regulating angiogenesis *(10,11)*. The balance between proangiogenic factors and antiangiogenic factors is considered to be critical in regulating angiogenic process. Thus, excess amount of proangiogenic stimuli leads to the progression of angiogenesis, but excess antiangiogenic milieu results in the resolution of angiogenic process. Various factors are known to induce angiogenesis (Table 1), but among those, proangiogenic capacity of VEGF, basic fibroblast growth factor (bFGF) and angiopoietins are well investigated and established to date. At present, there are many known antiangiogenic factors (Table 2) but these factors were already investigated extensively following the discovery of endostatin, a C-terminal domain of type XVIII collagen, exhibiting a potent antiangiogenic antitumor effect *(12)*. Subsequently various intrinsic antiangiogenic factors have been reported including angiostatin, restin, arresten, canstatin, and tumstatin *(13–18)*.

Endothelial cells differentiate from angioblasts in the embryo and from endothelial progenitor cells (EPC), multipotent adult progenitor cells or side-population cells in the adult bone marrow *(19,20)*. A portion of angiogenic factors are also known to recruit endothelial progenitor cells and facilitate the formation of neovessels *(21)*. Endothelial and hematopoietic progenitors share common markers and are affected by common signaling pathways. In fact, hematopoietic stem cells (HSCs) and leukocytes stimulate angiogenesis via releasing angiogenic factors or by transdifferentiation to endothelial cells *(22,23)*. The involvement of VEGF, placental growth factor (PlGF), angiopoietin-1, and inhibitor of differentiation (Id) in this process had been reported *(24,25)*. Angiogenesis is involved in a variety of physiological and pathological conditions, but initially it plays a critical role in the development of blood vessels and the cardiovascular system. Vasculogenesis, neovessel formation originating from EPCs, is critically involved in the development and is tightly linked to angiogenesis. Recent evidence indicates that EPCs contribute to angiogenic process also in the adult with ischemic, malignant, or inflammatory disorders. Therapeutic efficacy of EPC to stimulate vessel growth in ischemic disorders has been reported *(26,27)*. Angiogenesis and arteriogenesis refer to the sprouting and subsequent stabilization of these sprouts by mural cells. Angiogenesis is involved in physiological settings such as ovarian cycle, endometrium

Table 1
Proangiogenic Factors

VEGF	PDGF-BB
bFGF	Midkine
PlGF	Pleiotrophin
G-CSF	TGF-α, β
HGF	Follistatin
Angiopoietin-1/2	IL-3, 8
PD-ECGF	TNF-α
Angiogenin	Proliferin

Table 2
Antiangiogenic Factors

IFN-α,β,γ	Kringle 5
PF4	PEDF
Prolactin 16 kD frag.	IL-4, 10, 12
Angiostatin	Troponin-1
Endostatin	IP-10
Vasostatin	MIG
Arresten	Thrombospondin-1
Canstatin	Kininostastin
Tumstatin	TIMPs
Vasohibin	PEX
Angioarrestin	PAI
Endorepellin	Meth-1, 2

remodeling, and wound healing. Excessive angiogenesis is known to cause various disorders, and the best known are cancer, rheumatoid arthritis, psoriasis, and visual disturbance represented by diabetic retinopathy and retinopathy of prematurity *(11)*. Recent findings further added common disorders such as atherosclerosis, obesity, bronchial asthma, infectious disease, and endometriosis to the growing list of diseases associated with the involvement of angiogenesis. Several congenital and inherited diseases, such as DiGeorge syndrome, are also caused by abnormal vascular remodeling and angiogenesis. Insufficient angiogenesis and abnormal vessel regression lead to hypertension, preeclampsia, neurodegenaration as well as heart and brain ischemia.

3. Factors Involved in Regulating Angiogenesis

3.1. VEGF

The role of VEGF (also termed as VEGF-A) in regulating angiogenesis has been intensely investigated, and VEGF-signaling is critically involved in physiological as well as pathological angiogenesis represented by tumor growth *(28)*. A gene family of VEGF consists of VEGF-A, VEGF-B, VEGF-C, and PlGF *(28)*. VEGF-A is a key regulator of blood vessel growth, and VEGF-C and VEGF-D are involved in regulating lymphatic angiogenesis *(29)*. Inactivation of a single *Vegf* allele in mice resulted

in embryonic lethality between days 11 and 12 as a result of numerous developmental anomalies, defective vascularization in several organs, and reduction of nucleated red blood cells within the blood islands in the yolk sac, suggesting an essential role of VEGF in embryonic vasculogenesis and angiogenesis *(30,31)*. Mice deficient in VEGF-B or PlGF *(32)* did not exhibit any evident developmental abnormalities. VEGF induces the proliferation of endothelial cells in vitro *(28)*, and induces angiogenic response in vivo *(33)*. VEGF also induces survival of endothelial cells partly mediated via activating phosphatidylinositol (PI)-3 kinase-Akt pathway and by the increased production of antiapoptotic proteins Bcl-2 and A1 *(34,35)*. VEGF also promotes monocyte chemotaxis and induces vascular permeability, leading to inflammation and other pathological circumstances *(36,37)*. Consistent with its role in regulating vascular permeability, VEGF also induces fenestration of endothelial cells in some vascular beds *(38)*.

Four different splice variant isoforms of VEGF ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$) have been reported *(39,40)*. The properties of native VEGF (45 kDa) closely correspond with those of predominant isoform, $VEGF_{165}$, which is secreted but a significant fraction remains bound to the cell surface and ECM *(41)*. Expression of VEGF mRNA is induced under hypoxic condition in endothelial cells, mediated via hypoxia-inducible factor (HIF)-1 *(42)*. In addition, growth factors such as transforming growth factor (TGF)-α, TGF-β, insulin-like growth factor (IGF)-1, basic fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) upregulates the expression of VEGF mRNA *(28)*. Inflammatory cytokines such as interleukin (IL)-1α and IL-6 induce the expression of VEGF mRNA in synovial fibroblasts, leading to the development of joint lesions in rheumatoid arthritis.

VEGF binds to tyrosine kinase receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) *(28)*, and VEGF-C and VEGF-D bind to VEGFR-3 *(29)*. VEGF also bind to a family of co-receptors, the neuropilins.

3.1.1. VEGFR-1 (Flt-1)

Not only VEGF-A, but also VEGF-B and PlGF, which do not bind to Flk-1, bind to Flt-1. Upon binding to VEGF, Flt-1 undergoes weak tyrosine autophosphorylation *(43)*. A soluble extracellular domain of Flt-1 (sFlt-1) serves as a VEGF inhibitor *(44)*. Recent studies have revealed a synergistic effect of VEGF and PlGF in vivo as evidenced by impaired tumorigenesis in PlGF deficient mice *(32)*. Flt-$1^{-/-}$ mice die *in utero* between days 8.5 and 9.5 resulting primarily from excessive proliferation of angioblasts, suggesting that Flt-1 negatively regulates the activity of VEGF at least during early development *(45)*. The role of Flt-1 in hematopoiesis and recruitment of endothelial progenitors have also been demonstrated *(23)*. PlGF promoted collateral vessel growth in a model of myocardial infarction and treatment with anti-Flt-1 inhibited pathological conditions such as tumor, rheumatoid arthritis, and atherosclerosis suggest the involvement of Flt-1 in mediating angiogenic response *(25)*.

3.1.2. VEGFR-2 (KDR/Flk-1)

VEGFR-2, a high-affinity receptor of VEGF, serves as a major mediator of VEGF in inducing mitogenesis, angiogenesis, and vascular permeability *(46)*. Flk-1-null mice die *in utero* between days 8.5 and 9.5 resulting primarily from a lack of vasculogenesis and

failure to develop blood islands and organized blood vessels, suggesting the pivotal role of Flk-1 in developmental angiogenesis and hematopoiesis *(47)*. Upon binding of the ligand, Flk-1 undergoes dimerization, tyrosine phosphorylation, and phosphorylates several proteins in endothelial cells such as phospholipase C-γ, PI-3 kinase, Ras GTPase-activating protein, and the Src family of tyrosine kinases *(48,49)*. VEGF induces endothelial cell growth by activating the Raf-Mek-Erk pathway via protein kinase C, but not via Ras *(50)*. Activation of Flk-1 is required for the effect of VEGF in preventing apoptosis of endothelial cells through activating PI-3 kinase-Akt signaling pathway *(34)*.

3.1.3. Neuropilin

Neuropilin binds the semaphorin family and is implicated in neuronal guidance, and enhances $VEGF_{165}$-mediated chemotaxis *(51)*. It binds to $VEGF_{165}$ in an isoform-specific manner, presents $VEGF_{165}$ to VEGFR-2 enhancing the signaling mediated via VEGFR-2 *(51)*. Neuropilin-1-null mice exhibit embryonic lethality demonstrating the role of neuropilin-1 in the development of vascular system *(52)*.

3.2. Angiopoietins

The angiopoietin family comprises three structurally related proteins, Angiopoietin (Ang)-1, Ang-2, and Ang-3/4 *(53)*. Angiopoietin-1 consistently activates and phosphorylates Tie-2 receptor, but Ang-2 inhibits activation of Tie-2 and can even specifically block Ang-1-dependent phosphorylation *(54)*. Mice deficient in Ang-1 die at embryonic day 12.5, exhibiting some defects in vascular maturation, suggesting the important role of Ang-1/Tie-2 signaling in inducing endothelial-matrix interactions *(55)*. Mice deficient in Ang-2 die 2 wk after birth, exhibiting some defects in retinal vascularization and lymphatic function *(56)*. The expression of Ang-2 has been reported to be up-regulated by hypoxia, hypoxia inducible factor (HIF)-1α, VEGF, angiotensin-II, leptin, and other factors *(57,58)*. In contrast, very little is known about the regulatory mechanism of Ang-1. Ang-1 tightens vessels by affecting junctional molecules *(59)*, and is involved in the attachment of mesenchymal cells to endothelial-tube and differentiation to "pericytes," resulting in mature, "nonleaky" blood vessels *(55)*. Ang-1 binds to Tie-2 and promotes the firm attachment of pericytes *(55)*. The biological roles of angiopoietins are, however, pleiotropic and context-dependent. Ang-1 induces vessel growth in skin, ischemic limbs. and in some tumors *(60)*, possibly because of its effect on endothelial cell survival and mobilization of EPCs and HSCs *(61)*. However, Ang-1 also suppresses angiogenesis in tumors and the heart *(62,63)*. Ang-2, a natural antagonist of Ang-1, loosens the attachment of pericytes resulting in promoting sprouting angiogenesis in the presence of VEGF *(54)*. However, when insufficient angiogenic signals are present, Ang-2 causes endothelial cell death and vessel regression *(54)*.

3.3. Proteases

In the process of ECM remodeling during vessel sprouting, proteases such as urokinase plasminogen activator (uPA) and its inhibitor PAI-1, matrix metalloproteinases (MMPs) and tissue inhibitor of MMP, TIMPs, heparinases, and cathepsins play important roles *(64,65)*. HSPGs present in the basement membranes sequester proangiogenic growth factors such as VEGF and bFGF. Proteinases liberate matrix-bound proangiogenic factors,

thus facilitating sprouting angiogenesis. MMP-9 and MMP-2 are known to be required for the mobilization of the sequestered VEGF and thus the initiation of tumor angiogenesis *(66)*. Although MMP-9 does not effectively degrade perlecans or HSPGs, it degrades type IV collagen effectively, possibly disrupting the organization of basement membranes including HSPGs and perlecans, thus leading to the release of proangiogenic VEGF. Stromal and immune cells are known to produce MMPs *(67)*. Following matrix degradation and the release of VEGF, angiogenesis is initiated and tumors begin to grow resulting in the further recruitment of immune cells and fibroblasts. The effect of MMP-9 in releasing matrix-bound VEGF is potentially important at the early stage of tumor progression associated with angiogenic switch, it is not likely, however, to be required after the angiogenesis has been initiated *(68)*. Although insufficient breakdown of ECM prevents vascular endothelial cells from migrating into the interstitium, excessive degradation removes essential support and guidance for migrating endothelial cells, thus leading to sustained angiogenic response *(64)*. Following degradation of basement membranes by MMPs, cryptic domains of partially degraded collagens with proangiogenic activity become exposed *(69)*. On the other hand, proteinases are known to liberate matrix-bound or matrix comprising angiogenesis inhibitors such as endostatin *(12)*, canstatin *(16)*, arresten *(15)*, tumstatin *(17,70)*, restin *(14)*, endorepellin *(71)*, and thrombospondin (TSP)-1, resulting in the resolution of angiogenesis. Expression of MMPs increases as the tumors grow larger, and the levels of MMPs in serum and urine are known to be useful indicators of tumor progression and metastasis. Degradation of basement membranes by MMPs therefore acts as both a positive (at the early stage) and a negative (at the middle to late stage) regulator of tumor angiogenesis *(68,72)*. Interestingly, a domain of MMP-2, PEX, can inhibit the interaction of MMP-2 and αvβ3 integrin, thus leading to the suppression of tumor growth *(73)*.

3.4. Ephrins

The Eph family of receptor tyrosine kinases and corresponding ligands ephrins had originally been identified to determine embryonic patterning and neuronal targeting *(74)*. EphA receptors bind the ephrinA ligands; EphB receptors bind ephrinB ligands, with the exception of EphA4 binding to both ephrins *(75)*. Mice deficient in ephrinB2 or EphB4 die during embryogenesis with severe cardiovascular defects, suggesting the involvement in the primary capillary network remodeling and patterning in embryonic vasculatures *(76,77)*. Reciprocal expression pattern of ephrinB2 and EphB4 in arterial and venous endothelial cells, suggests that they might interact at the arterial-venous interface *(78)*. EphA2 was observed in tumor-associated vascular endothelial cells, ephrinA1 was detected in tumor as well as endothelial cells and soluble EhpA2-Fc exhibited anti-angiogenic and anti-tumor effect, suggesting the involvement in tumor angiogenesis *(79)*.

3.5. Integrins

Integrins, cell surface receptors for extracellular matrix, exist as heterodimers of α and β subunits, forming at least 25 different combinations of receptors with distinct and overlapping specificity for ECM proteins *(80)*. Integrins transmit bidirectional "outside-in" or "inside-out" signals *(4)*. The αvβ3 integrin is predominantly expressed on endothelial cells in tumor vasculature *(81)*, and antibodies against αvβ3 or αvβ5 integrin

or RGD peptide recognized by these receptors efficiently blocked angiogenesis in tumors and retina *(7,82,83)*. Antibodies against αvβ3 interfered with bFGF-induced angiogenic effect, and antibodies against αvβ5 interfered with VEGF-induced angiogenic effect *(83)*. Genetically engineered mice lacking β3 or β5-integrin are fertile and viable, and αv-integrin deficient mice showed extensive angiogenesis *(8,84,85)*. Mice lacking one or both of these integrins (β3 or β5-integrin) exhibited enhanced tumor growth and angiogenesis *(85)*, suggesting the possibility that these integrins serve as negative regulators of angiogenesis. Previous reports have demonstrated the *trans*-dominant negative regulation of both the α5β1 and α2β1 integrin by $\alpha_{IIb}\beta3$ integrin *(86)*. Apparent discrepancy on the function of αvβ3 and αvβ5 integrins might be mediated via *trans*-dominant inhibition of other proangiogenic integrins (α5β1, α1β1, or α2β1 integrin) in endothelial cells *(4)*. Thus, reagents directed at αvβ3 or αvβ5 integrin might indirectly inhibit proangiogenic function of other integrins such as α5β1 integrin, instead of directly inhibiting proangiogenic activity of αvβ3 or αvβ5 integrin. Inhibitory action of αvβ3 integrin on the level of Flk1, resulting in reduced responses to VEGF *(87)*, further support the function of αvβ3 integrin as a negative regulator of angiogenesis.

3.6. Cadherin

Vascular endothelial cadherin (VE-cadherin), a transmembrane glycoprotein located at adherent junctions of endothelial cells, plays a pivotal role in maintaining vascular integrity. Cytoplasmic tail of VE-cadherin interacts with β- and γ-catenin, which in turn promote the anchorage to actin cytoskeleton. Targeted inactivation or truncation of the VE-cadherin gene resulted in embryonic lethality resulting from altered vascular remodeling and impairment of VEGF-mediated endothelial cell survival and angiogenesis *(88)*, suggesting that VE-cadherin is required for proper angiogenic process. In fact, antibodies against VE-cadherin blocked angiogenesis and tumor growth *(89)*.

4. Mechanisms of Tumor Angiogenesis

Primary tumors and metastases initially exist as small avascular masses, and avascular *in situ* carcinomas may exist for months or years, consequently remaining as a small mass of a few cubic millimeters. Subsequently, some tumor cells switch to the angiogenic phenotype and induce the ingrowth of new capillary blood vessels to allow further tumor growth *(10,90)*. Many primary epithelial tumors, originally separated from underlying blood vessels by basement membranes, develop in this manner, requiring the degradation of basement membranes to access the vasculatures. Expansion of tumor mass occurs not only by increased perfusion of blood through the tumor, but also by paracrine stimulation of growth factors and matrix proteins produced by the newly formed capillary endothelium *(11)*. The switch to the angiogenic phenotype depends on a net balance of proangiogenic factors and antiangiogenic factors. Proangiogenic factors include bFGF, VEGF, and other factors secreted from tumor cells or infiltrating macrophages or mobilized from the ECM. Increased levels of proangiogenic factors accompanied by decreased levels of antiangiogenic factors are required for the 'angiogenic switch'.

In nonangiogenic *in situ* tumors or in dormant micrometastases, proliferation of tumor cells continues, but is balanced by high rates of apoptosis of tumor cells *(91)*. The

angiogenic switch in human tumors are driven by: (1) angiogenic oncogenes which up-regulate proangiogenic factors and/or down-regulate antiangiogenic factors; (2) hypoxia leading to the activation of HIF-1 resulting in the activation of VEGF; (3) fibroblasts in the tumor bed producing proangiogenic factors; and (4) endothelial progenitor cells derived from bone marrow which migrate into tumor vasculatures *(92)*. Once the angiogenic switch is turned on, neovessels converge on the dormant *in situ* tumors and tumor cells cluster around each microvessel. The angiogenic switch is associated with a marked decrease in tumor cell apoptosis.

The interaction of tumor cells with host blood vessels has been described in association with VEGF and Ang-2 *(53)*; tumor cells initially home in on and grow by co-opting existing blood vessels, and start off as well-vascularized small tumors *(93)*. The host blood vessels regress in response to the inappropriate co-option as a result of elevated autocrine expression of Ang-2, starve the tumor, and render tumors avascular and hypoxic. The co-opted blood vessels undergo apoptosis and the tumor becomes avascular and hypoxic, leading to marked induction of VEGF from tumor cells. In the presence of elevated levels of VEGF, regression of destabilized co-opted blood vessels will stop, and subsequent sprouting angiogenesis from these blood vessels will allow for the survival and growth of the tumor. The microenvironment with high levels of Ang-2 may explain why tumor vasculatures fail to mature, and tend to be leaky and hemorrhagic *(53)*.

Antiangiogenic therapy can inhibit endothelial cell proliferation in a tumor bed or increase endothelial cell apoptosis. Endothelial cells in the tumor bed have significantly higher proliferation rates than quiescent endothelium and they should be more susceptible to cytotoxic agents. Several chemotherapeutic agents, in fact, inhibit angiogenesis in addition to inducing tumor cell death. For example, paclitaxel, which inhibits microtubule polymerization, inhibits proliferation and migration of endothelial cells, and inhibits tumor growth *(94)*. Continuous administration of chemotherapeutic agents such as cyclophosphamide or vinblastine at a lower dosage than the usual maximum tolerated dose (antiangiogenic schedule) resulted in complete tumor regression and long-term survival in experimental animal models *(95)*.

5. Inhibitors of Angiogenesis (*see* Table 3)

5.1. Endostatin

Type XVIII collagen is a triple helical molecule harboring several heparan sulfate side-chains, thus classified as a major proteoglycans of epithelial and vascular basement membranes *(96,97)*. The C-terminal globular noncollagenous (NC) domain contains the antiangiogenic fragment, endostatin *(12)*. This antiangiogenic domain is separated from an upstream trimerization region by a protease-sensitive hinge *(96)*. Upon cleavage within this hinge region, endostatin will be released into circulation and tissues *(98)*. Type XVIII is known to be involved in the normal development of vasculature in the retina, and a splice mutation in human type XVIII collagen has been associated with Knobloch syndrome, a disease with insufficient development of retinal vasculatures leading to retinal degeneration and blindness *(99)*.

Type XV collagen, sharing homology with type XVIII collagen *(100)*, is a disulfide-bonded proteoglycan containing chondroitin sulfate side-chains. These collagens are

Table 3
Endogenous Basement Membrane Derived Angiogenesis Inhibitors

Angiogenesis inhibitor	Parent protein	Domain	Receptors	Inhibitory activities on EC
Endostatin	α1 chain of type XVIII collagen	NC1	Glypicans, flk-1, α5β1-integrin	Proliferation, migration, tube formation, survival, tumor growth
Restin	α1 chain of type XV collagen	NC10	Unknown	Proliferation, migration, tumor growth
Tumstatin	α3 chain of type IV collagen	NC1	αvβ3-integrin	Proliferation, tube formation, survival, protein translation, tumor growth
Arresten	α1 chain of type IV collagen	NC1	α1β1-integrin	Proliferation, migration, tube formation, survival, tumor growth
Canstatin	α2 chain of type IV collagen	NC1	α3β1-integrin	Proliferation, migration, tube formation, survival, tumor growth
α6(IV)NC1	α6 chain of type IV collagen	NC1	Unknown	Proliferation
Endorepellin	Perlecan	Domain V	α2β1-integrin	Migration, tube formation, survival, blood vessel growth *in vivo*

multiplexin subclass of nonfibrillar collagens containing an N-terminal NC domain, triple-helical regions with multiple interruptions by NC domains, and C-terminal NC1. Type XV collagen is widely distributed in several basement membranes of various tissues, including vascular basement membranes *(101)*. Studies from mice deficient in type XV collagen revealed the important structural role of this collagen in stabilizing skeletal muscle cells and microvessels *(102)*. Similar to type XVIII collagen, the C-terminal NC domain, termed as restin, was shown to possess anti-angiogenic activity *(14,103)*.

Endostatin, a 20 kDa C-terminal NC1 domain of type XVIII collagen, possesses potent antiangiogenic activity *(12)*. Endostatin was first isolated from the conditioned medium of murine hemangioendothelioma cells. Endostatin inhibits endothelial cell proliferation, migration and tube formation in vitro *(104–106)* and possesses potent inhibitory effect on tumor growth in vivo *(12,107)*. The inhibitory effects of endostatin on the expression of VEGF in tumor cells and on vascular permeability had been reported *(108)*. Endostatin also blocks VEGF-mediated proangiogenic signaling via direct interaction with flk-1 *(109)*. Recent reports have demonstrated the therapeutic potential of endostatin in nonneoplastic disorders with involvement of angiogenic process such as rheumatoid arthritis and proliferative diabetic retinopathy *(110,111)*. These reports have also demonstrated the antiangiogenic activity of synthetic peptide derived from N-terminal domain of human endostatin in vitro and in vivo *(112)*.

Cell surface receptors for endostatin had been extensively studied recently. Rehn et al. reported that recombinant human endostatin interacted with α5- and αv-integrins on the surface of human endothelial cells, and demonstrated the functional significance of this interaction *(113)*. More recently, interaction of endostatin with α5β1 integrin in association with cell surface HSPGs and caveolin have been reported *(114,115)*. The interaction of endostatin with α5β1 integrin led to the activation of caveolin-associated Src tyrosine kinase, p190RhoGAP phosphorylation, followed by inactivation of RhoA, and disassembly of actin stress fibers and focal adhesion, the mechanism potentially involved in the inhibitory effect of endostatin on endothelial cell migration *(115)*. Sudhakar et al. reported that human endostatin binding to α5β1 integrin led to the inhibition of the activation of focal adhesion kinase and subsequent inhibition of mitogen-activated protein kinases (MAPKs) including extracellular signal-regulated kinase (ERK)-1/2 and p38MAPK, resulting in the inhibition of endothelial cell migration *(116)*. In addition, heparan sulfate glycosaminoglycans, or glypicans, were reported to bind to endostatin, and this interaction was important for mediating endostatin's antiangiogenic activities in vitro *(117)*. The requirement of E-selectin, an inducible leukocyte adhesion molecule specifically expressed on endothelial cells, for the antiangiogenic activity of endostatin has also been reported *(118)*. Suppressive effect of endostatin on the expression of Id1 and Id3, essential proteins in regulating cell growth and differentiation, and the transcription factor Ets-1 which promotes angiogenesis via inducing several target genes such as MMP-1, MMP-3, MMP-9 and β3 integrin, had been recently reported *(119)*.

5.2. Tumstatin

Type IV collagen, the most abundant constituent of the basement membranes, is expressed as six distinct α-chains, namely, α1–α6 *(120)*, assembles into triple helices,

and forms organized networks. Network assembly of type IV collagen is essential for structural integrity and biological functions of basement membranes. In general, type IV collagen possesses the ability to promote cell adhesion, migration, differentiation, and cell growth *(121)*. Although type IV collagen is found only in basement membranes in normal conditions, it is associated with organ fibrosis and accumulates in tumor interstitium. The α-chains consist of N-terminal cysteine-rich 7S domain, the middle collagenous triple helical domain with Gly-X-Y repeats interrupted by short NC sequences, and a C-terminal globular NC1 *(2)*. Superstructures self-associate from triple helical monomers to form either dimers (via interactions of NC1 domains) or tetramers (via interaction of 7S domains), with 56 possible combinations *(122)*. The α1(IV) and α2(IV) chains are ubiquitously distributed in human basement membranes and heterotrimers composed of 2 α1(IV) and 1 α2(IV) chains are predominant *(123)*. In contrast, the localization of the other four isoforms is tissue- and organ-specific *(124,125)*. The distribution of the α3(IV) chain is limited to certain basement membranes, such as glomerular basement membrane, several basement membranes of the cochlea, ocular basement membrane of the anterior lens capsule, Descemet's membrane, ovarian and testicular basement membrane *(126)*, and alveolar capillary basement membrane *(125,127)*. This chain is absent from epidermal basement membranes of the skin and the vascular basement membrane of liver *(125)*.

The NC1 domain of type IV collagen is considered to play a crucial role in the assembly of type IV collagen to form trimers, and thus influence basement membrane organization and modulation of cell behavior *(2,121)*. Petitclerc et al. demonstrated the potent biological activity of recombinant α2(IV), α3(IV), and α6(IV) NC1 domains to inhibit angiogenesis and tumor growth *(18)*.

We identified the pivotal role of recombinant human α3(IV)NC1 in inhibiting the proliferation of capillary endothelial cells and blood vessel tube formation, and also in inducing endothelial cell specific apoptosis *(17)*. We named this domain as "umstatin"(for its unique property of causing "tumor-stasis"), to add another member to the family of endogenous inhibitors of angiogenesis derived from larger proteins, such as angiostatin, endostatin and restin *(12–14)*. Tumstatin further inhibited in vivo neovascularization in matrigel plug assays and suppressed tumor growth of human renal cell carcinoma (786-O) and prostate carcinoma (PC-3) in xenograft mice models *(17)*. Endothelial cell apoptosis of tumor vasculatures was induced in tumstatin-treated mice with PC-3 tumors.

Goodpasture syndrome is an autoimmune disease characterized by pulmonary hemorrhage and/or rapidly progressive glomerulonephritis *(127)*. These symptoms are caused by the disruption of glomerular and pulmonary alveolar basement membrane through immune injury associated with autoantibody against α3(IV) NC1 *(127)*. The most probable disease-related pathogenic autoepitope was identified in the N-terminal portion *(128,129)* and was further confined to be within the N-terminal 40 amino acids *(130,131)*. Truncated tumstatin (tum-1) lacking N-terminal 53 amino acids encompassing the pathogenic Goodpasture autoepitopes, was fully active in inhibiting tumor growth and angiogenesis, and another deletion mutant tum-2, consisting of 124 amino acids in the N-terminal half portion of tumstatin possessed equivalent antiangiogenic activity specific to endothelial cells *(17)*. In contrast, deletion mutant tum-3 consisting of 120 amino acids in the C-terminal half portion of tumstatin, failed to inhibit

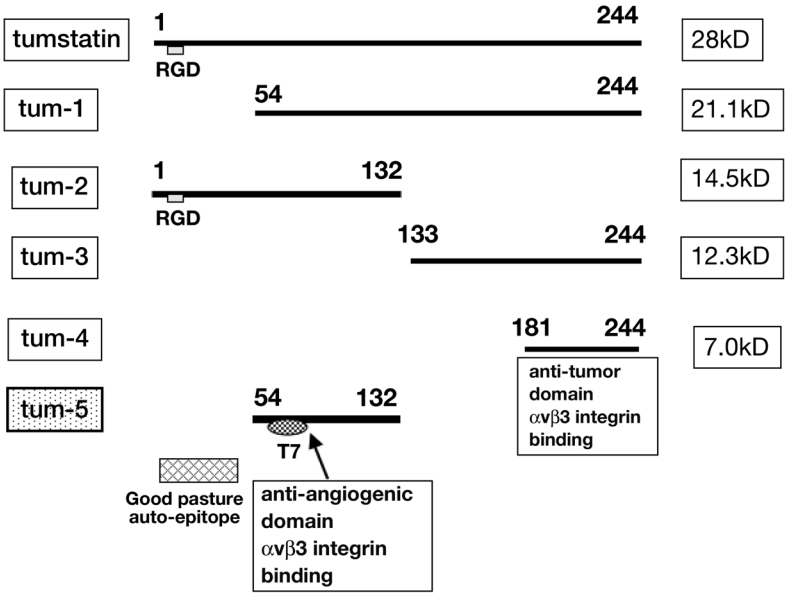

Fig. 2. Anti-angiogenic domain of tumstatin. Tumstatin, a 244-kDa domain derived from C-terminal NC1 domain of α3 chain of type IV collagen, possesses antiangiogenic tum-5 domain that binds to αvβ3 integrin in an RGD-independent manner. This domain does not include Goodpasture autoepitope and the core antiangiogenic domain is located at T7 peptide domain. C-terminal tum-4 domain possesses antitumor activity with binding to αvβ3 integrin in an RGD-independent manner.

angiogenic responses in endothelial cells. These results collectively suggested the localization of antiangiogenic domain within the amino acids 54–124 of tumstatin, which differs from the Goodpasture auto-epitope (*see* Fig. 2).

Previously, the α3(IV)NC1 domain had been shown to bind and inhibit the growth of melanoma cell lines in vitro via amino acids 185-203 of α3(IV)NC1 domain *(132)*. Deletion mutant tum-4 consists of 64 amino acids in the C-terminus of tumstatin, which includes the 185–203 peptide region. Although tum-4 did not inhibit the proliferation of endothelial cells, it inhibited the proliferation of melanoma cells (WM-164). The antitumor activities of tumstatin were thus considered to localize in distinct region of tumstatin, different from antiangiogenic domain *(17)*.

The NC1 domain of α1 and α2 chain of type IV collagen (arresten and canstatin, respectively) also possesses antiangiogenic activity. Arresten, the 26-kDa NC1 domain of the α1 chain of type IV collagen, inhibits endothelial cell proliferation, migration, tube formation, and in vivo neovascularization using matrigel plug assay. Arresten also inhibits the growth of two human xenograft tumor models in nude mice and the development of tumor metastases. The antiangiogenic activity of arresten was mediated via interaction with cell surface proteoglycans and the α1β1 integrin on endothelial cells *(15)*. Canstatin, an endogenous 24-kDa fragment of human α2 chain of type IV collagen inhibits endothelial cell proliferation, migration and murine endothelial cell tube formation. In addition, canstatin potently induced apoptosis of endothelial cells, without exhibiting any effects on nonendothelial cells. Treatment with canstatin did not

inhibit the activation of ERK1/2. The proapoptotic effect of canstatin on endothelial cells was associated with a down-regulation of the antiapoptotic protein, FLIP. Canstatin also suppressed in vivo growth of tumors in two human xenograft mouse models with decreased CD31-positive tumor vasculatures *(16)*. The cell surface receptors of canstatin are considered as αvβ3 and α3β1 integrin. Canstatin inhibited the activation of PI3-kinase/Akt resulting in the induction of apoptosis dependent upon signaling events transduced through Fas membrane death receptors *(133)*.

5.2.1. Tumstatin Binds to αvβ3 Integrin

Within the N-terminal portion of tumstatin, there is a RGD sequence (amino acids 7–9). In general, the RGD sequence is considered as an important binding site for αvβ3 integrin. Previous studies have identified the 185–203 amino acid sequence of α3(IV)NC1 as a ligand for αvβ3 integrin and responsible for the associated antitumor cell property *(134)*. We found distinct additional RGD independent αvβ3 integrin binding site within 54–132 amino acids of tumstatin *(135)*. This site does not include the RGD sequence, and, as described above, this site is not essential for inhibition of tumor cell proliferation but necessary for the antiangiogenic activity. A fragment of tumstatin containing 54–132 amino acids could bind both endothelial cells and melanoma cells, but could only inhibit proliferation of endothelial cells, with no effect on tumor cell proliferation. Another fragment of tumstatin containing the 185–203 amino acid (tum-4) could bind both endothelial cells and melanoma cells, but could only inhibit the proliferation of melanoma cells. These results may suggest the involvement of additional cell-specific ligand receptor interactions for exerting cell-specific antiproliferative effects. The presence of cyclic RGD peptides did not affect the αvβ3 integrin-mediated antiangiogenic activity of tumstatin, although significant inhibition of endothelial cell binding to vitronectin was observed. Thus, the two distinct RGD-independent αvβ3 binding sites on tumstatin mediated two separate antitumor activities, suggesting unique αvβ3 integrin mediated mechanisms governing the two distinct anti-tumor properties of tumstatin *(135)*.

5.2.2. Tum-5 Domain

In order to directly demonstrate the antiangiogenic activity of the putative 54–132 amino acids of tumstatin (tum-5 domain), the recombinant tum-5 was produced in *Escherichia coli* and *Pichia pastoris*. Recombinant tum-5 proteins were not recognized by antisera obtained from patients with Goodpasture syndrome, excluding the possibility that these recombinant proteins might induce an autoimmune disorder. Tum-5 specifically inhibited proliferation and caused apoptosis of endothelial cells with no significant effect on nonendothelial cells such as melanoma cell line (WM-164) or prostate carcinoma cell line (PC-3) *(136)*. Tum-5 also inhibited tube formation of endothelial cells on matrigel and induced G1 cell cycle arrest of endothelial cells. In addition, antiangiogenic effect of tum-5 was demonstrated in vivo using both a Matrigel plug assay in C57BL/6 mice, and human prostate cancer (PC-3) xenografts in nude mice. Tum-5 at 1 mg/kg significantly inhibited growth of PC-3 tumors in association with a decrease in CD31 positive tumor vasculatures. Cell attachment assays revealed that tum-5 could bind to αvβ3 integrin in an RGD-independent manner, and was thus independent of vitronectin binding. Competition proliferation assays utilizing soluble αvβ3

integrin protein to compete αvβ3 integrin receptors on the cell surface revealed that the antiproliferative effect of tum-5 was reversed dose-dependently with an increasing amount of αvβ3 soluble protein, suggesting the requirement of tum-5-αvβ3 integrin interaction in antiangiogenic effect of tum-5 *(136)*. These results suggested the potential role of αvβ3 integrin in negative regulation of angiogenesis by binding to tum-5.

5.2.3. Tumstatin Peptide

Reduction and alkylation of tumstatin and tum-5 failed to alter the antiangiogenic activity in vitro and in vivo, suggesting this activity was independent of disulfide bond requirement *(137)*. Therefore, five overlapping synthetic peptides were designed so that they would cover the tum-5 domain. Among these peptides, only the T3 (69–88 amino acids) and T7 peptide (74–98 amino acids) inhibited proliferation and induced apoptosis specifically in endothelial cells. T3 peptide, similar to tumstatin and the tum-5 domain, could bind and exert antiangiogenic effects via the αvβ3 integrin in an RGD-independent manner. Restoration of a disulfide bond between two cystines within the T3 peptide did not affect on the antiangiogenic activity of T3 peptide. Antiangiogenic effect of the peptides was further confirmed in vivo using a Matrigel plug assay in C57BL/6 mice. These results suggested that the antiangiogenic activity of tumstatin was localized to a 25 amino acid region of tumstatin (T7 peptide) *(137)*.

5.2.4. Tumstatin Inhibits Protein Synthesis in Endothelial Cells

Apoptosis, programmed cell death, is regulated in part at the level of protein synthesis, and is generally associated with inhibition of cap-dependent protein translation *(138–140)*. Because tumstatin selectively stimulates apoptosis of endothelial cells via binding to αvβ3 integrin, the potential effects of tumstatin peptides on protein synthesis have been examined. Tum-5, T3, and T7 peptide inhibited protein synthesis in endothelial cells, but not in other nonendothelial cells. In contrast, endostatin did not exhibit similar effects on protein synthesis. Tumstatin peptides exhibited endothelial cell-specific inhibitory effect on cap-dependent translation, and this inhibitory effect was not observed in β3-integrin-deficient endothelial cells *(141)*.

In many cell types, ligand binding to integrin induced phosphorylation of focal adhesion kinase (FAK) and subsequent activation of various signaling molecules *(142,143)*. For instance, phosphorylated FAK interacts with and activates phosphatidylinositol 3-kinase (PI3-knase) and protein kinase B (PKB/Akt), leading to cell survival *(142,144)*. In fact, inhibition of PI3-kinase in endothelial cells has been shown to repress protein synthesis *(145)*. Tumstatin peptides inhibited phosphorylation and activation of PI3-kinase and phosphorylation of Akt in endothelial cells *(141)*. Akt activates Rapamycin/FKBP-target 1 protein (RAFT1), also known as mammalian target of rapamycin (mTOR), which in turn phosphorylates eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP1) *(146,147)*. Unphosphorylated 4E-BP1 forms complex with eIF4E and inhibits cap-dependent translation *(148)*. In fact, stimulation of cells with growth factors induces phosphorylation of 4E-BP1, dissociation of 4E-BP1 from eIF4E, leading to the induction of protein translation *(147,148)*. Tumstatin peptides suppressed mTOR kinase activity, phosphorylation of 4E-BP1, and dissociation of 4E-BP1 from eIF4E, thus resulting in inhibition of cap-dependent translation. In contrast, tumstatin peptides failed to inhibit phosphorylation of ERK1/2 upon vitronectin

attachment or stimulation with VEGF in endothelial cells. Overexpression of constitutive active Akt resulted in reversal of tumstatin peptide-induced inhibition of cap-dependent translation. These results suggested the possible inhibitory effects of tumstatin peptides on protein synthesis of endothelial cells through negative regulation of mTOR signaling. Alternatively, interaction of tumstatin peptide with αvβ3 integrin may induce negative regulatory signals counteracting growth factor-initiated cell survival signals (*see* Fig. 3).

5.2.5. Endogenous Tumstatin Inhibits Angiogenesis and Tumor Growth

In addition to the antiangiogenic effect of exogenously added tumstatin, the endogenous functions of tumstatin have been reported by Hamano et al. *(70)*. Mice deficient in α3 chain of type IV collagen, a precursor of tumstatin, exhibited normal pregnancy, development, and wound healing process, but had accelerated pathological angiogenesis and tumor growth. Although tumstatin inhibited angiogenesis in mice with the physiological expression levels of β3 integrin, inhibitory effect of tumstatin was not observed in β3 integrin-null mice, suggesting the requirement of β3 integrin in tumstatin's antiangiogenic effect *(70)*. Treatment of basement membranes with MMP-9 generated tumstatin fragments in vitro. Other MMPs—MMP-2, 3, and 13—could release tumstatin fragment from basement membranes, although the efficiency was significantly less than MMP-9. In fact, mice deficient in MMP-9 exhibit significantly decreased circulating blood concentrations of tumstatin compared with wild-type mice. The growth of Lewis lung carcinoma tumors was similar in both MMP-9(+/+) and MMP-9(−/−) mice until the tumors reached 500 mm^3, but the tumors on MMP-9(−/−) mice exhibited accelerated tumor growth afterwards. Intravenous administration of tumstatin to raise the concentration of tumstatin to normal levels resulted in the retardation of tumor growth similar to the level of wild-type mice, suggesting the importance of physiological levels of tumstatin in regulating tumor growth *(70)*. MMP-9 has been implicated as a positive regulator of angiogenic switch, leading to reduced tumor growth and tumor vasculatures upon genetic ablation of MMP-9 in the early stage of tumor growth *(66)*. However, pharmacological inhibition of MMP-9 resulted in acceleration of tumor growth *(72)*. Whereas MMP-9 may mediate the angiogenic switch leading to the initial burst of tumor growth, it also suppresses tumor growth by generating endogenous inhibitors of angiogenesis such as tumstatin *(70)*. The physiological endogenous anti-angiogenic role of TSP-1 and endostatin via binding to CD36 and α5β1 integrin, respectively, had also been recently demonstrated *(149)*.

5.2.6. Application of Tumstatin Peptide on Non-neoplastic Disorder

Diabetic nephropathy is complicated in 30–40% of patients with type 2 diabetes and is the most common pathological disorders predisposing end-stage renal diseases (ESRD) in Japan and in the Western World *(150)*. Hyperglycemia is involved in the progression of diabetic nephropathy and early alterations in diabetic nephropathy include glomerular hyperfiltration, glomerular and tubular epithelial hypertrophy, and the development of microalbuminuria *(151)*. These early alterations are followed by the development of glomerular basement membrane thickening, the accumulation of extracellular matrix components in the mesangium as well as in the interstitium, and the increase of urinary albumin excretion, eventually leading to glomerulosclerosis and

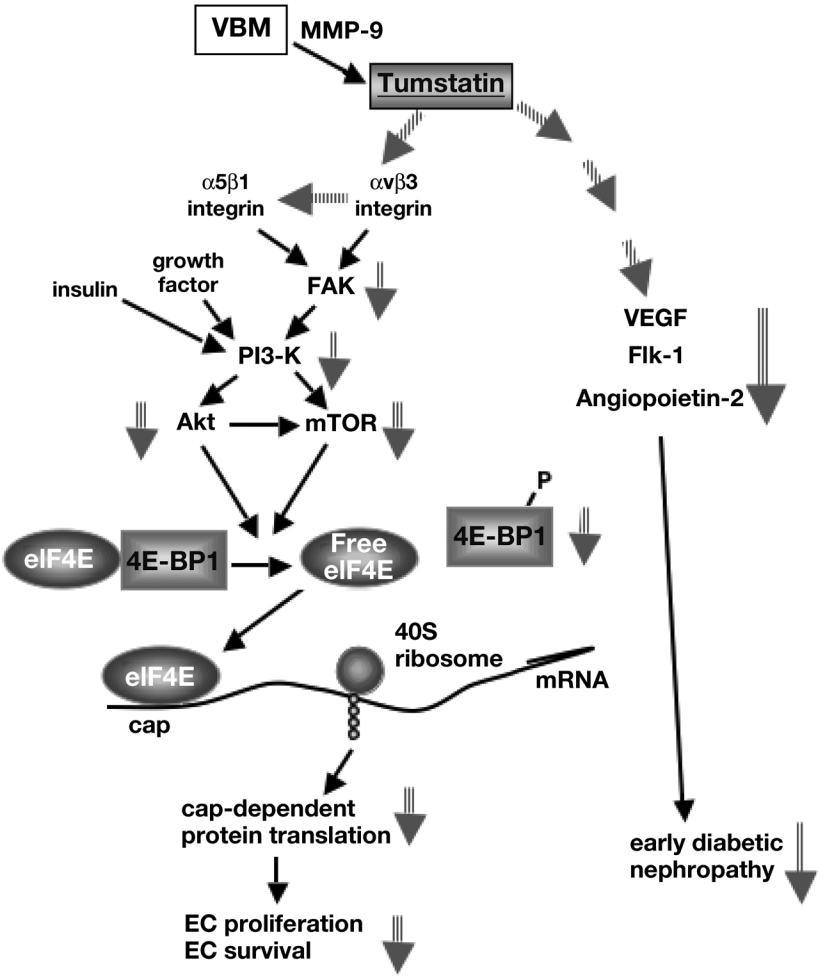

Fig. 3. Schematic of the mechanism of action of tumstatin in inhibiting angiogenesis. Proteases (e.g., MMP-9) degrade vascular basement membrane (VBM) to generate tumstatin, which subsequently binds to αvβ3 integrin on endothelial cells. Tumstatin negatively regulates the pathway that includes focal adhesion kinase (FAK), phosphatidylinositol 3-kinase (PI3-K), Akt, mammalian target of rapamycin (mTOR), eukaryotic translation initiation factor (eIF4E), and eIF4E-binding protein 1 (4E-BP1), resulting in the inhibition of protein synthesis and proliferation of endothelial cells (EC). Upon binding to αvβ3 integrin, tumstatin may also cause transdominant inhibition of angiogenic signals from α5β1 and α2β1 integrin. Tumstatin derived peptide suppresses the renal expression of VEGF, angiopoietin-2 and flk-1, leading to the suppression of early alterations in diabetic nephropathy. The effects of tumstatin are shown by striped arrows.

progressive loss of renal function *(152,153)*. Recent reports by Yamamoto et al. implicated the therapeutic efficacy of the tumstatin peptide in the early stage of diabetic nephropathy via down-regulating renal expression of VEGF, flk-1, and Ang-2 *(154)*, further emphasizing the potential biological functions of tumstatin on various disorders involving angiogenic process (*see* Fig. 3).

5.3. Endorepelin

5.3.1. Perlecan

Perlecan is a major HSPG of basement membranes and vascular and avascular ECM, involved in regulating cell growth, differentiation, cell adhesion, and the development of blood vessels, cartilage, and the nervous system *(155)*. Perlecan-null mutations result in early embryonic lethality accompanied by severe cephalic and cartilage abnormalities *(156)*. Embryos that survive initially usually develop later malformations of the cardiovascular system *(157)*. In humans, two rare skeletal disorders, dyssegmental dysplasia silver-handmaker type (DSSH) and Schwartz-Jampel syndrome (SJS) are caused by mutations of genes encoding perlecan *(158,159)*. Perlecan is considered to exert proangiogenic effects, because it binds to and protect growth factors from degradation and it interacts with adhesion molecules *(160)*.

5.3.2. Endorepellin, an Antiangiogenic C-Terminal Fragment of Perlecan

Perlecan, a C-terminal 85-kDa domain V of perlecan inhibits endothelial cell migration, tube formation, and growth of blood vessels *(71)*. Endorepellin is suspected of inhibiting angiogenesis in a dominant-negative fashion, endothelial cells secrete perlecan. Endorepellin reversibly alters the actin cytoskeleton of endothelial cells, potentially associated with its effects on cell motility and other alterations in cell morphology *(161)*. The interaction of endorepellin with $\alpha 2\beta 1$ integrin triggers a unique signaling pathway that causes an increase in the second messenger camp;activation of protein kinase A and FAK; transient activation of p38 mitogen-activated protein kinase and heat shock protein 27, followed by a rapid down-regulation of the latter two proteins; and ultimately disassembly of actin stress fibers and focal adhesions associated with the inhibitory effect on endothelial cell migration and angiogenesis *(161)*. Collectively, a family of C-terminal fragment of endogenous matrix proteins (endostatin, tumstatin and endorepellin) harbors potent antiangiogenic effects potentially generated via processing by proteases.

5.4. Anti-VEGF Reagents

Based on the well-known role of VEGF in promoting angiogenesis and tumor growth, therapeutic strategies to block the biological effect of VEGF had been developed and, to date, clinically tested on patients with refractory tumors. The therapeutic effect of neutralizing anti-VEGF or VEGF receptor antibody, small molecule inhibitor of tyrosine kinase receptor of VEGF in experimental tumor models has been reported *(79)*.

Observation of proteinuria upon treatment with anti-VEGF antibody or soluble flt-1 (an antagonist of VEGF) has been reported in experimental animal models *(162)* and clinical trials, suggesting the possible side effect on glomerular filtration barrier.

6. Conclusion

Here, we described on the biological roles of angiogenic response in developing tumors and the mediators and inhibitors of angiogenesis, emphasizing the endogenous inhibitors of angiogenesis derived from basement membrane proteins. Further publications on the role of angiogenesis in developing cancer implicate the significance of this research field and its potential to lead to the development of novel therapeutic approaches to halt and regress tumor growth. Investigation on the biological function of endogenous angiogenesis

inhibitors may provide insight into the regulatory mechanism of endothelial cells and other vascular cell components in the setting of various pathological disorders.

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