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The Matrix Reloaded: New Insights from Type IV Collagen Derived Endogenous Angiogenesis Inhibitors and their Mechanism of Action

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Abstract

Angiogenesis, the process of neovascularization from parent blood vessels, is a prerequisite for many physiological and pathological conditions that is regulated by a balance between the levels of endogenous angiogenic stimulators and matrix reloaded angiogenic regulators. Several non-collagenous carboxy terminal end domains in α chains of type IV collagen matrix reloaded molecules selectively interact with proliferating endothelial cells by binding to distinct integrins and regulate intracellular signaling and inhibit angiogenesis. This review will focus on the current understanding of extra cellular matrix type IV collagen reloaded endogenous angiogenesis inhibitors and their mechanism of action.

Introduction

An emerging area of research in cell biology is the discovery of extra cellular matrix (ECM) that is reloaded with proteolytic fragments from the basement membrane, which exert powerful anti-angiogenic or anti-tumarogenic activities. Angiogenesis, the process by which new blood vessels are originated from parent blood vessels, is essential in normal development and also pathological conditions such as cancer (solid tumor growth), rheumatoid arthritis and diabetes retinopathy (Marneros and Olsen, 2001; Ortega and Werb, 2002; Sottile, 2004; Sudhakar, 2007). In cancerous conditions, tumor cells need new blood vessels for nourishment, local growth and escape to remote organs sites through blood circulation, a process called metastasis. Without angiogenesis, within the tumor, tumor cells cannot stay alive for longer time. For this reason, matrix reloaded endogenous inhibitors of angiogenesis, which might not produce the same sort of resistance and intolerance as exogenous compounds, might be important candidates for anticancer therapy, either by themselves or in combination with other inhibitors that are used routinely in the clinics.

The first discovered ECM reloaded endogenous angiogenic inhibitory molecule is endostatin, a 20-kDa fragment of heparan sulfate proteoglycan derived from type XVIII collagen non-collagenous (NC1) domain is best characterized among endogenous angiogenic inhibitors. Since initial discovery of endostatin as endogenous angiogenesis inhibitor, there has been an outburst in anti-angiogenic research and in the number of endogenous molecules that are known to promote or inhibit angiogenesis (Iozzo and San Antonio, 2001; Marneros and Olsen, 2001; Ortega and Werb, 2002; Schenk and Quaranta, 2003; Sottile, 2004; Sudhakar, 2007) (Figure 1). Several endogenous anti-angiogenic protein fragments were discovered that were derived from ECM constituents. Although they are structurally different they have remarkable similari-

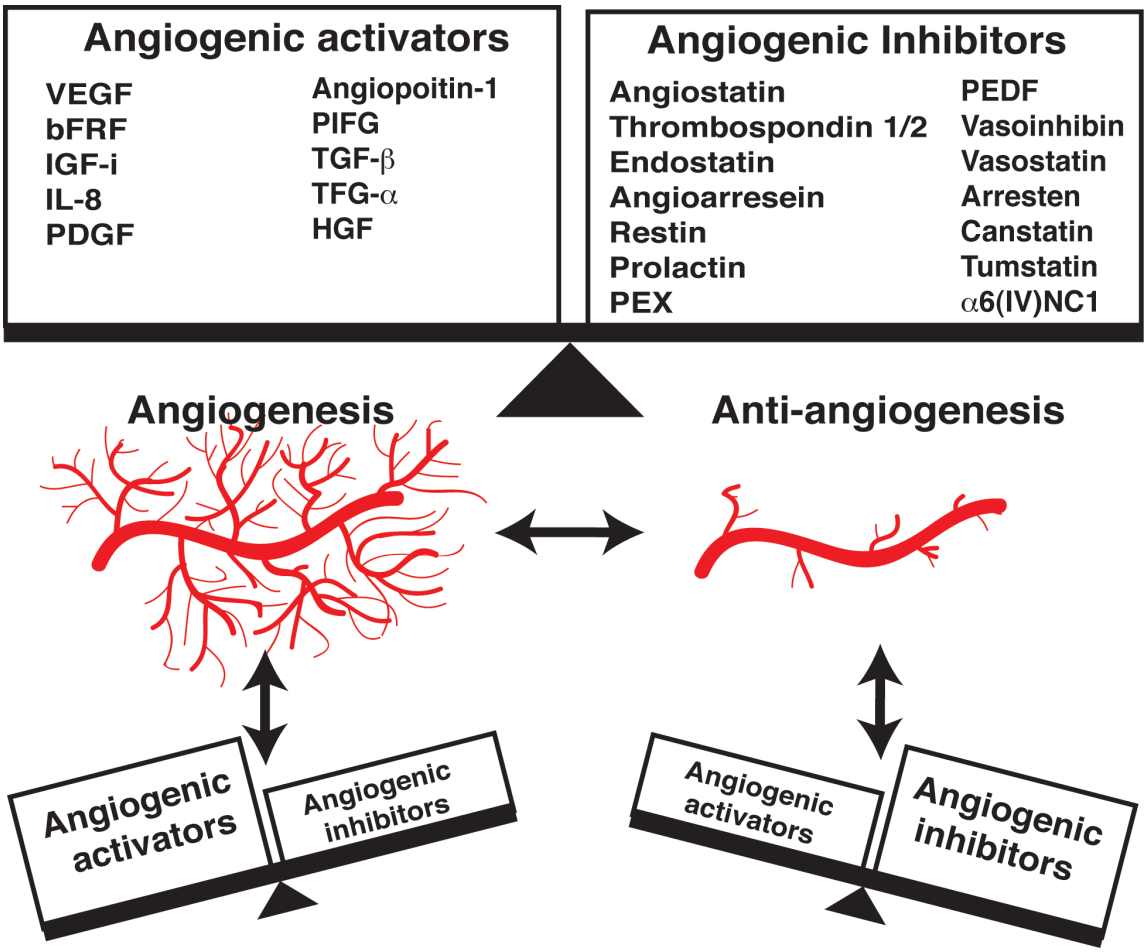

Figure 1: The angiogenic balance between angiogenic activators and angiogenic inhibitors regulate vascular homeostasis. Angiogenesis under physiological and pathological conditions is associated with up-regulation of angiogenic factors and/or down-regulation of angiogenic inhibitors. Up-regulation of angiogenic inhibitors and/or down-regulation of angiogenic activators may be associated with impaired neovascularization capacity. VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; IGF-I, insulin-like growth factor-I; IL-8, interleukin-8; PDGF, platelet-derived growth factor; PlGF, placenta growth factor; TGF-α and β, transforming growth factor-α and β.

ties that must be more than coincidental. More recently, type IV collagen α1, α2, α3 and α6 chain carboxy terminal NC1 domains were demonstrated to be anti-angiogenic. These molecules that are needed to assemble vascular basement membrane (VBM) to maintain the integrity of blood vessels and to prevent the leakage of fluids and loss of proteins can do the opposite actions under the different circumstances (called matrix reloaded); that is, they have both pro-angiogenic and anti-angiogenic activities (Iozzo and San Antonio, 2001; Nakamura and Matsumoto, 2005; Sudhakar and Boosani, 2008). Therefore, matrix reloaded endogenous type IV collagen derived angiogenic inhibitors mechanism of action are of high significance and is updated in this review.

Structural Integrity and Biological Insights of Type IV Collagen Matrix

The discovery of endostatin as an endogenous angiogenesis inhibitor initiated researchers to hasten their investigation and to find other ECM derived components that are antiangiogenic and anti-tumorogenic with the potential to modulate angiogenesis. Type IV collagen is ECM-specific and abundant collagen with various isoforms whose network assembly is essential for the structural integrity and biological function of basement membranes (Myllyharju and Kivirikko, 2004). Type IV collagen has six α chains and exists in atleast three heterotrimeric triple helical forms $[\alpha1(IV)]_2\alpha2(IV)$, $[\alpha3(IV)]_2\alpha4(IV)$ and $[\alpha5(IV)]_2\alpha6(IV)$ (Hudson et al., 1993; Hudson et al., 2003; Kalluri, 2003). Each α(IV) chain has a long, collagenous domain with Gly-X-Y repeats (X and Y represent any amino acids other than glycine, but are often proline and hydroxyproline, respectively) interrupted by short NC sequences, an N-terminal cysteine-rich 7S domain and a C-terminal NC1 domain of 230 amino acids. These superstructures self-associate from triple helical monomers to form either dimers (via NC1–NC1 interactions) or tetramers (via 7S-domain in-

teractions) with 56 possible combinations (Kalluri, 2003; Sudhakar and Boosani, 2008).

Type IV collagen molecules that contain different α(IV) chains have different cellular interactions and localizations. Furthermore, proteolysis of ECM might expose cells to new, cryptic interaction sites within type IV collagen (Kalluri, 2003). The α1(IV) and α2(IV) chains are ubiquitous and $[\alpha1(IV)]_2\alpha2(IV)$ heterotrimers are predominant, whereas α3(IV) chain expression is limited. Interestingly, in many patients with Alport's syndrome (either X-linked autosomal recessive hereditary kidney disease that is characterized by a defect in glomerular BM) mutations in α5(IV) chain led to loss of α3(IV) chain which indicates that their expression is co-dependent (Cosgrove et al., 1996; Hudson et al., 2003). Type IV collagen is highly conserved, structurally and functionally in vertebrates and in invertebrates (Blumberg et al., 1987; Netzer et al., 1998). Normally, type IV collagen promotes cell adhesion, differentiation, migration and growth (Sarras et al., 1993). In C. elegans two genes encoding type IV collagen have been identified, emb-9 (α1-like) and let-2 (α2-like). Mutations in these two genes might cause either inhibited embryonic development or variably severe temperature-sensitive mutants (Myllyharju and Kivirikko, 2004). In Drosophila, type IV collagen genes DCG1 and viking are involved in muscle attachment and pericardin is involved in development of heart. A mutation in α1(IV) was identified with perinatal cerebral hemorrhage stroke, porencephaly, endoplasmic reticulum stress and genetically modifiable ocular dysgenesis (Gould et al., 2007; Gould et al., 2005; Gould et al., 2006). In humans or in mice, no specific disease is yet linked to mutations in α2(IV) chains.

Type IV Collagen Matrix Reloaded Angiogenesis Inhibitors and their Mechanism of Action

Arresten: Arresten (α1(IV)NC1), a 26-kDa protein is derived from C-terminal NC1 domain of α1 chain type IV collagen by proteases (Boosani and Sudhakar, 2006; Colorado et al., 2000; Sudhakar et al., 2005). Not much is known about this angiogenesis inhibitor but its anti-antiangiogenic actions are presumably mediated through α1β1 integrins (Sudhakar et al., 2005). Integrin α1β1 is a collagen binding receptor that also binds to other basement membrane components such as laminin (Keely et al., 1995; Zutter and Santoro, 1990). Blocking of α1β1 integrin interactions with ECM inhibits angiogenesis indicating that the integrin α1β1 acts as a pro-angiogenic receptor (Senger et al., 1997). Among the integrin receptors for collagen, α1β1 integrin activates the Ras/Shc mitogen activated protein kinase (MAPK) pathway promoting cell proliferation (Senger et al., 1997; Sudhakar et al., 2005). Arresten binds to α1β1 integrin in a collagen type IV dependent manner and mediates its anti-angiogenic and pro-apoptotic functions and inhibits angiogenesis by inhibiting endothelial cell proliferation, migration and tube formation (Boosani et al., 2009; Nyberg et al., 2008; Sudhakar et al., 2005). Significant halt in pathological angiogenesis and tumor growth was reported in α1 integrin knockout mice (Pozzi et al., 2000). Whereas arresten had no effect in α1 integrin null endothelial cells, on the contrary it significantly inhibited proliferation of wild type mouse endothelial cells, which confirms the significance of integrin α1β1 mediated signaling of arresten (Sudhakar et al., 2005). Arresten inhibits phosphorylation of FAK/Ras/Raf/MEK1/2 and p38 MAPK when endothelial cells are plated on collagen type IV matrix (Figure 2). Similar inhibition of MAPK signaling was not observed with arresten treatment to α1 integrin null endothelial cells (Sudhakar et al., 2005). Downstream to FAK, Akt/PKB plays an important role in endothelial cell survival signaling. Arresten does not inhibit Akt or phosphatidyl-3-kinase (PI3 kinase) phosphorylation suggesting that arresten regulates migration of endothelial cells in an Akt-independent manner (Sudhakar et al., 2005).

Interestingly proteolysis of ECM reloaded arresten inhibits hypoxia (lack of oxygen) inducible factor alpha (HIF-1α) and VEGF expression in endothelial cells in an α1β1 and FAK/Ras/Raf/MEK1/2 and p38 MAPK dependent manner (Sudhakar et al., 2005) (Figure. 2). HIF-1α is an oxygen-dependent transcriptional factor which plays prominent roles in tumor angiogenesis (Semenza, 2003). HIF-1α regulates cellular responses to physiological and pathological hypoxia, and studies so far demonstrate that HIF-1α is a potential target to inhibit tumor angiogenesis (Unruh et al., 2003). HIF-1α transcriptionally regulates VEGF expression in hypoxic cells and promotes angiogenesis in solid tumors (Carmeliet et al., 1998; Kung et al., 2000; Sudhakar et al., 2005). These findings suggest that HIF-1α is a prime target for anticancer therapies and this hypoxic inhibitory activity might be exploited for antiangiogenic therapy in the treatment of different solid tumor cancers, but more pre-clinical laboratory studies are needed.

In addition, recently we also reported that arresten inhibits VEGF mediated angiogenesis by promoting apoptosis in different endothelial cells. Arresten activates caspase-3/PARP [Poly (ADP-ribose) polymerase] activation and negatively impacts FAK/p38-MAPK phosphorylation via down-regulation of anti-apoptotic Bcl-2 and

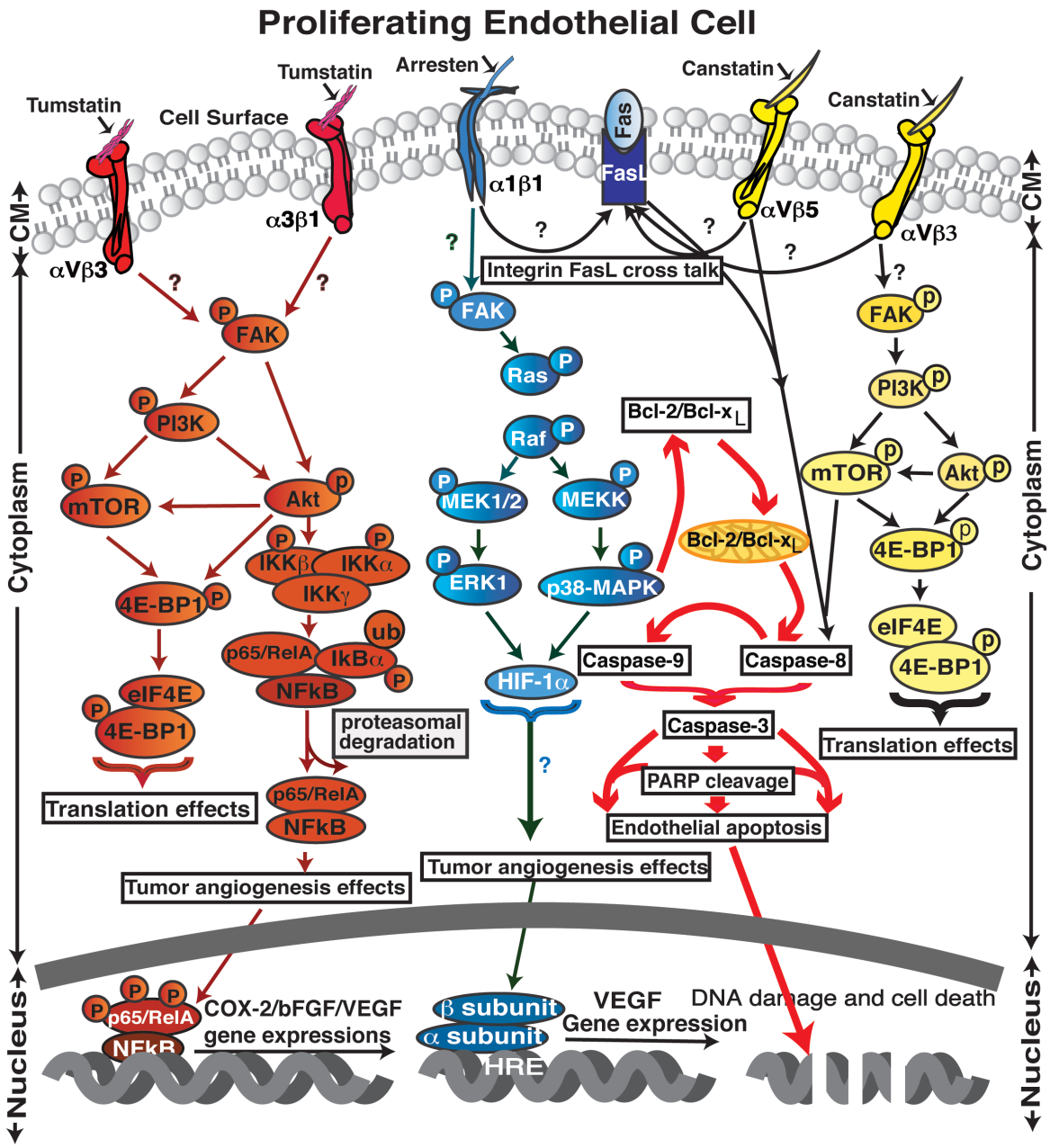

Figure 2: Schematic illustration of distinct antiangiogenic and anti-tumorogenic signaling mediated by different type IV collagen matrix reloaded molecules. Tumstatin, arresten and canstatin interact with αVβ3/α3β1, α1β1 and αVβ3/αVβ5 integrins, respectively, to inhibit the phosphorylation of focal adhesion kinase (FAK). **Tumstatin:** It binds to αVβ3 and 3β1 integrins and inhibits the pathway that includes phosphorylation of FAK, PI3-K, Akt, mTOR, 4E-BP1 and eIF4E to decrease endothelial cell protein synthesis and proliferation. In addition tumstatin also inhibits NFκB mediated signaling in hypoxic conditions leading to the inhibition of COX-2, VEGF and bFGF expressions, resulting in inhibition of hypoxic tumor angiogenesis. **Arresten:** It binds to α1β1 integrin and inhibit phosphorylation FAK, causes inhibition of Ras, Raf, extra cellular signal related kinase 1 (ERK1) and p38 MAPK pathways that leads to inhibition of HIF-1α and VEGF expression resulting in inhibition of endothelial cell migration, proliferation and tube formation. In addition arresten initiates two apoptotic pathways, involving activation of caspase-9 and -8, leading to activation of caspase-3 and PARP cleavage. (a) Arresten activates caspase-3 directly through inhibition of FAK/p38-MAPK/Bcl-2/Bcl-x_L and activation of caspase-9; (b) Integrin α1β1 cross talk with Fas-L through mitochondrial pathway and leads to activation of caspase-8 and-3 in proliferating endothelial cells. **Canstatin:** It binds to αVβ3/αVβ5 integrins and inhibits two apoptotic pathways, involving activation of caspase-8 and casoase-9, leading to activation of caspase-3. Canstatin activates procaspase-9 not only through inhibition of the FAK/PI3K/AKT pathways but also by integrins cross talking mitochondrial pathway through Fas-L dependent caspase-8 activation leads to endothelial cell apoptosis. CM represents cell membrane.

Bcl-xL expressions with no effect on pro-apoptotic Bax expression leading to endothelial cell death (Boosani et al., 2009; Nyberg et al., 2008). These Bcl-family members are considerably involved in the balance of pro and anti-apoptotic signals at the mitochondrial level. The balance of pro and anti-apoptotic protein expression determines whether cells undergo apoptosis (Cory et al., 2003). These studies demonstrate that activation of apoptotic signaling in proliferating endothelial cells is sufficient to inhibit new blood vessel formation. These results are in consistent with earlier reports that anti-angiogenic and anti-tumorogenic activity of arresten is mediated via integrin cross talk with a cell-intrinsic apoptotic pathway (Boosani et al., 2009) (Figure 2). The endothelial specific inhibitory actions of ECM reloaded arresten may be of benefit in the treatment of a variety of diseases with a neovascular component.

Canstatin: Proteolytic degradation of type IV collagen liberates a 24-kDa protein fragment from C-terminal NC1 domain of α2 chain, called canstatin [α2(IV)NC1] that was reported to inhibit tumor angiogenesis and tumor growth (Kamphaus et al., 2000; Petitclerc et al., 2000). Canstatin binds to endothelial and tumor cells in an αVβ3 and αVβ5 integrin dependent manner (Magnon et al., 2005). Canstatin competes with type IV collagen of ECM for cell surface integrin binding and reverses the proliferatory and migratory effects induced by cell-ECM interactions. Thus, αVβ3 and αVβ5 integrins appear to mediate the antiangiogenic and pro-apoptotic actions of canstatin (Magnon et al., 2005; Panka and Mier, 2003). Canstatin inhibits the growth of many tumors in mouse xenograft models and histological studies revealed decreased CD31 positive vasculature. Canstatin binds to αVβ3 and αVβ5 integrins and inhibits endothelial migration and proliferation (Kamphaus et al., 2000; Magnon et al., 2005; Panka and Mier, 2003; Roth et al., 2005). Moreover, these events are mediated by inhibiting phosphorylation of FAK, Akt, mammalian target of rapamycin (mTOR), eukaryotic initiation factor 4E binding protein-1 (4E-BP1) and ribosomal S6 kinase (Panka and Mier, 2003). Canstatin binds to αVβ3 and αVβ5 integrins and initiates two apoptotic pathways that includes (a) activation of caspase-8 and -9 and leads to activation of caspase-3 and (b) activation of caspase-8 by downregulation of Flip levels (Magnon et al., 2005; Narazaki and Tosato, 2006). Upregulation of Fas/Fas ligand triggers not only cell death directly through caspase-3 activation but also indirectly through mitochondrial damage via activation of caspase-9 within the apoptosome (Magnon et al., 2005; Magnon et al., 2008; Wang et al., 2008). On the other hand, phosphorylated FAK/PI3K is known to inactivate the mitochondrial apoptotic pathway by inhibition of caspase-9 (Figure. 2). Canstatin directly activates procaspase-9 through inhibition of FAK/PI3K pathway and amplifies the Fas-dependent pathway in mitochondria. An intravitreous injection of canstatin causes selective apoptosis in endothelial cells resulting in inhibition of neovascularization when given prior to the onset of new vessel sprouting (Magnon et al., 2005; Roth et al., 2005). Importantly, when canstatin was given after neovascularization had already developed, it caused the new vessels to regress.

In tumor cells canstatin activates caspase-3 only in the mitochondrial pathway (Figure. 2). Canstatin suppresses tumor angiogenesis in different mice xenograft models and also laser induced choroidal nonvascular (CNV) in mice, indicating it as a strong antiangiogenic agent in the choroid and as a therapeutic candidate for treatment of CNV in age related macular degeneration (Lima et al., 2006). These results demonstrate that canstatin binds to αVβ3 and αVβ5 integrins and inactivates FAK down stream signaling leading to inhibition of cell proliferation and migration, and thus leading to activation of apoptosis and inhibition of tumor angiogensis.

Tumstatin: Physiological proteolysis of α3 chain type IV collagen by MMP-9 results in tumstatin (α3(IV)NC1) a 28-kDa C-terminal NC1 domain (Hamano et al., 2003). The anti-angiogenic activity of tumstatin was observed with pharmacological doses of this protein associated with inhibition of protein synthesis in endothelial cells (Maeshima et al., 2002; Sudhakar et al., 2003). The expression of tumstatin is down-regulated in renal carcinoma growth (Xu et al., 2009). Inhibition of melanoma and fibrosarcoma cell migration using tumstatin peptide (185-205 amino acids) was reported with a decrease in expression of membrane-bound metalloproteinase (MT1-MMP) and activated MMP-2 (Han et al., 1997). Tumstatin inhibits tube formation in mouse aortic endothelial cells embedded in Matrigel plugs and inhibits tumor growth in different mouse models [renal cell carcinoma (786-O), CT26 (colon adenocarcinoma), prostate carcinoma (PC3), lewis lung carcinoma (LLC), human lung cancer (H1299), human prostate cancer (DU145), human fibrosarcoma (HT1080), teratocarcinoma (SCC-PSA1) and lymphatic metastasis in an orthotopic oral squamous cell carcinoma model] (Boosani et al., 2007; Chung et al., 2008; Hamano et al., 2003; Maeshima et al., 2000b; Miyoshi et al., 2006; Pedchenko et al., 2004; Petitclerc et al., 2000; Sudhakar and Boosani, 2008). In addition tumstatin gene therapy

enhanced anti-tumor effect of gemcitabine in murine models (Yao et al., 2005). But what integrins are engaged in these antiangiogenic and anti-tumorogenic actions of tumstatin? It is clear that different integrins are key targets of tumstatin to mediate its antiangiogenic and anti-tumorogenic actions. Tumstatin interacts with endothelial cell via αVβ3 integrin in a vitronectin and RGD independent fashion (Maeshima et al., 2000a). Integrin αVβ3 interacts with tumstatin through two separate regions. The first region 54-132 amino acids is involved in the antiangiogenic activity, whereas the second region 185-203 amino acids is involved in anti-proliferative activity in cancer cell lines (Boosani et al., 2007; Eikesdal et al., 2008; Maeshima et al., 2000a; Pedchenko et al., 2004). These results substantiate exact regulatory sub-domains in tumstatin controlling adhesion and proliferation in various cell types. The functional specificity of these two sub domains from tumstatin in cancer or endothelial cells is very interesting. Indeed published 3D crystal structure of type IV collagen NC1 domain reveals N and C-homologous sub-domains (Than et al., 2002). The major difference between these sub-domains for each chain is in the region from residues 86-95 in the N-sub-domain and 196-209 in the C-sub-domain. These regions overlap two sequences that were previously identified to be having antiangiogenic and anti-proliferative effects in cancer cells (Borza et al., 2006). Tum-1, a tumstatin peptide, gene delivery into hepatocellular carcinoma cells suppressed tumor growth by inhibiting angiogenesis (Goto et al., 2008). Tumstatin or its peptides interaction with integrins seems to be involved in the disruption of contacts between endothelial or tumor cells leading to apoptosis in these cells, but the mechanism of apoptosis activation is still not clear. Tumstatin also binds to endothelial cells through other integrins α6β1 and αVβ5, but the significance of these integrins interaction is not yet clear (Boosani et al., 2007; Maeshima et al., 2000a).

Tumstatin induces endothelial cell apoptosis by interacting with integrin αVβ3 and inhibits VEGF adhesion to matrix, and this effect was overturned by integrin αVβ3 blocking antibody. The antiangiogenic activity of tumstatin is conferred by its interaction with integrin αVβ3 and inhibiting activation of focal adhesion kinase (FAK), phosphatidylinositol 3-kinase (PI-3K), serine/threonine kinase (Akt/protein kinase B), mammalian target of rapamycin (mTOR) and prevents dissociation of eukaryotic translation initiation factor 4E (eIF4E) from 4E binding protein (4E-BP1) leading to the inhibition of Cap-dependent translation in proliferating endothelial cells (Maeshima et al., 2002; Sudhakar et al., 2003) (Figure. 2). PTEN/Akt pathway dictates the direct αVβ3 integrin dependent growth inhibitory action of T7 peptide of tumstatin in glioma cells in-vitro and *in-vivo* (Kawaguchi et al., 2006). Furthermore, these results conforms a specific function for integrins in mediating endothelial cell specific inhibition of cap-dependent translation, signifying a possible specific mechanism of tumstatin. Whereas α3β1 integrins bind to C-terminal region 185-203 residues associated with antiangiogenic and anti-tumorogenic activity (Borza et al., 2006), 17426256}. These results compare earlier findings that tumstatin binds to α3β1 integrin and transdominantly inhibits expression of αVβ3 integrin (Boosani et al., 2007). Interestingly recent studies clearly show tumor suppressive action of tumstatin or its peptide (T3; C-terminal end comprising amino acid residues 133-244 region of the tumstatin) that directly inhibits growth of glioma cells (Kawaguchi et al., 2006). In addition a cyclopeptide derived from tumstatin (YSNSG) was also shown to inhibit human melanoma cell proliferation (Thevenard et al., 2006).

Recently we discovered that tumstatin inhibits hypoxia induced cyclo-oxygenase-2 (COX-2) expression in endothelial cells via FAK/Akt/NFkB (nuclear transcription factor-kappa B) pathway leading to decreased tumorangiogenesis and tumor growth in a α3β1 integrin dependent manner (Figure 2). In addition to COX-2 inhibition, the down stream VEGF and bFGF protein expression was also inhibited upon tumstatin treatment to endothelial cells (Boosani et al., 2007). Moreover, researchers have confirmed that inhibition of COX-2 signaling serves as a therapeutic benefit in different cancer models and potential target for tumor angiogenesis (Boosani et al., 2007; Harris, 2002). These findings indicate that there may be several targets for the inhibitory effects of tumstatin in tumor-angiogenesis. All these studies support that the antiangiogenic and anti-tumorogenic activity of tumstatin is mediated through αVβ3 and α3β1 integrins. Astonishingly, aberrant tumor growth and tumor vasculature is observed in tumstatin knockout mice, demonstrating that tumstatin is playing a prominent role in pathological angiogenesis to decrease tumor progression (Hamano and Kalluri, 2005; Hamano et al., 2003). However in a controlled physiological angiogenic process such as wound healing, tumstatin does not affect the physiological angiogenesis and /or neovascularization. The amino acids residues 185-191 that contain the sequence CNYYSNS in which the YSNS motif forms a β-turn have the same anti-tumor activity compared to intact tumstatin (Thevenard et al., 2006). Furthermore, replacing Cys185 with Asp, but not Met prevents the anti-tumor activity, which indicates the importance of a sulfur atom in its structure and function (Thevenard et al., 2006; Thevenard et

al., 2009). Although tumstatin is efficient in reducing tumorangiogenesis and /or tumor neovascularization, its exact role needs to be deciphered.

Signaling Convergence and Complementarities Between Arresten, Canstatin and Tumstatin

The carboxy terminal localization might be necessary for different proteases to access and liberate the antiangiogenic domains from type IV collagen that are embedded in the ECM. These protease-generated domains exert their effects by binding to different integrins that help modulate how cells interact with and are affected by matrix components. The functional integrin receptor for each anti-angiogenic molecule liberated from type IV collagen are different and specific; α1β1 for arresten, αVβ5/αVβ3 for canstatin and αVβ3/α3β1/αVβ5/α6β1 for tumstatin. This indicates that the mechanism of integrin receptors engagement and downstream signaling mechanism might also be different and specific for matrix reloaded endogenous angiogenesis inhibitors, although some common intracellular signaling components vary between these angiogenic inhibitors. Arresten and canstatin seem to exert their effects on the endothelial cytoskeleton that is essential for the cells to migrate and form capillary like structures. Canstatin and tumstatin function by inhibiting protein synthesis and growth and survival of proliferating endothelial cells. Arresten, canstatin and tumstatin can directly affect the integrin receptor complement and have functional consequences (Table 1). Finally, these three antiangiogenic domains are different structurally, which explains why they interact with different integrins and have different effects on angiogenesis. By limiting our focus to these three antiangiogenic domains, we hope to highlight similarities that might also apply to other undiscovered antiangiogenic ECM fragments and further our understanding of angiogenesis inhibition with potential therapeutic implications against cancer.

Significance of Bioavailability of ECM Reloaded Endogenous Angiogenesis Inhibitors

At present there are several ECM derived endogenous angiogenesis inhibitors in pre-clinical trails including type IV collagen derived arresten, canstatin and tumstatin (Table 1). These endogenous matrix reloaded molecules were found available in patients at nano molar levels. Owing to their strong anti-angiogenic and anti-tumorogenic actions and their availability in meager quantities, these endogenous molecules have been synthesized, expressed and purified from different heterologus systems to increase their bioavailability and to treat patients with neovascular diseases such as cancer, rheumatoid arthritis and diabetic retinopathy.

Concluding Remarks

Angiogenesis is a dynamic process. *In-vivo*, endogenous molecules have been identified that stimulate, inhibit and modulate this process. A common, revolutionary theme has gradually emerged for endogenous, ECM derived anti-

Angiogenesis inhibitors name	Arresten	Canstatin	Tumstatin
Origin	*α1 chain Type IV collagen NC1 domain*	*α2 chain Type IV collagen NC1 domain*	*α3 chain Type IV collagen NC1 domain*
Generated	Matrix metalloprotensae-9	Matrix metalloprotensae-9 and-2	Matrix metalloprotensae-9,-3 and -13
Integrin binding	α1β1,?	αVβ3, αVβ5,?	αVβ3, α3β1, α6β1, αVβ5,?
Proliferation	Inhibits endothelial proliferation	Inhibits endothelial proliferation	Inhibits endothelial proliferation
Migration	Inhibits endothelial migration	Inhibits endothelial migration	No effect on endothelial migration
Tube formation	Inhibits endothelial tube formation	Inhibits endothelial tube formation	Inhibits endothelial tube formation
Antiangiogenic activity	Inhibits endothelial migration and promoted apoptosis	Inhibits protein synthesis by activating endothelial apoptosis	Inhibits endothelial growth and protein synthesis
Pathways affected	FAK/MAPK/HIF1α and FasL/Bcl-2/Bcl-xL mediated signaling	FAK/Akt/PI3/mTOR/eIF-4E/4E-BP1 and FasL mediated signaling	FAK/Akt/PI3K/mTOR/eIF-4E/4E-BP1 and NFkB/COX-2 mediated signaling

Table 1: Type IV collagen matrix reloaded angiogenesis inhibitors and their mechanisms of action.

angiogenic molecules: C-terminal end fragments of many type IV collagen matrix exert their inhibitory effects (at least in part) by interacting with integrins. It is feasible that proteases that degrade ECM or VBM and enable endothelial cell migration during angiogenesis also generate C-terminal anti-angiogenic peptides. In pathological conditions such as cancer, anti-angiogenic signals might be overwhelmed by pro-angiogenic (growth factors) signals that are produced by tumor cells.

Arresten canstatin and tumstatin are antiangiogenic and are also potent anti-tumorogenic molecules that are derived from the C-terminal end of type IV collagen seem to have diverse complementary effects. In addition to direct functional interactions among these type IV collagen NC1 domains, the antiangiogenic activity of these NC1 domains is modulated by partially overlapping signal transduction pathways. However, till date several questions remain to be answered and numerous issues need to be solved like how these antiangiogenic NC1 domains are reloaded from matrix? Are these domains regulating different signaling mechanisms? Is it significant that these domains are C-terminally derived? Are there any other cellular receptors for these domains in addition to cell surface integrins? To what extent does the overlap in signaling affect the anti-angiogenic potencies of the individual domains *in-vivo*? Are the physiological blood levels of these domains generated from type IV collagen (all are in nano-molar range) sufficient to exert anti-tumor activity? Is the presence of these domains in the circulating blood a mere indicator of turnover of the ECM reloaded? Should their *in-vivo* activity be viewed as a pharmacological effect? Most importantly, will any of these collagen type IV derived endogenous angiogenesis inhibitors be a successful clinical tool in the fight against cancer and other vascular diseases such as choroidal neovascularization of age related macular degeneration? Whatever the final results, by recognizing the commonalities of type IV collagen ECM derived endogenous anti-angiogenic domains, we should gain invaluable, clinically significant insights for optimal therapeutic interventions.

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