Published in final edited form as:
Microvasc Res. 2007 ; 74(0): 85–89. doi:10.1016/j.mvr.2007.05.005.

Type IV collagen-derived Angiogenesis Inhibitors

Thomas M. Mundel[1] and **Raghu Kalluri**[1,2,3]

[1]Division of Matrix Biology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02115

[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02215

[3]Harvard-MIT Division of Health Sciences and Technology, Boston, MA 02215

Abstract

The concept of anti-angiogenesis therapy was introduced by Judah Folkman in 1972 and since then, a plethora of pro- and anti-angiogenic factors have been identified. In the recent years it has become clear that angiogenesis, the formation of new capillaries from a pre-existing capillary network, is highly regulated by the action of pro- and anti-angiogenic factors. In the healthy adult organisms the "*angiogenic-switch"* is likely turned *"Off"*, i. e. anti-angiogenic factors are likely counteracting the pro-angiogenic factors resulting in a non-angiogenic state. Angiogenesis is encountered during wound healing processes, the female menstrual cycle and endometrial remodeling, as well as during embryonic development and organ growth. In the pathological setting, angiogenesis plays an important role in different diseases like rheumatoid arthritis, psoriasis, macular degeneration, diabetic retinopathy, and tumor growth. In this regard, recent studies have described several endogenous inhibitors of angiogenesis, with a subset derived from extra cellular matrix (ECM) proteins. This review will particularly focus on the type IV collagen-derived angiogenesis inhibitors Arresten, Canstatin and Tumstatin.

Keywords

ECM; angiogenesis inhibitor; type IV collagen NC1; tumor

The diffusion capacity for oxygen and nutrients in tissues is limited to about 150-200 μm, therefore most cells of an organism are located within a radius of 150-200 μm around a capillary (Carmeliet and Jain, 2000). Hence, tumor growth beyond 1 mm^3 of volume is not optimally possible without neo-vascularization (Carmeliet and Jain, 2000; Folkman, 1972; Folkman, 1995; Folkman and Kalluri, 2003; Hanahan and Folkman, 1996). Moreover

Address for Correspondence: Dr. Raghu Kalluri Associate Professor of Medicine Harvard Medical School Division of Matrix Biology, DANA 514 Beth Israel Deaconess Medical Center 330 Brookline Avenue Boston MA 02215 Tel: 617.667.0445 Fax: 617.975.5663 rkalluri@bidmc.harvard.edu.

tumors metastasize into other organs via the newly formed blood vessels (Carmeliet and Jain, 2000; Sauer and Deissler, 2003). In general, two types of neo-vascularization are recognized in the body. Blood vessel formation that occurs during embryonic development via the contribution of blood vessel precursor cells called angioblasts is called vasculogenesis. Angiogenesis, the formation of new blood vessels from a pre-existing capillary network, can occur in a sprouting dependent or non-sprouting dependent manner. During sprouting angiogenesis, endothelial cells degrade the vascular basement membrane (VBM), invade into the surrounding tissue and proliferate in response to angiogenic factors. Non-sprouting angiogenesis, also known as intussusception, occurs as a longitudinal division of an existing vessel. The long-term thinking that vessel formation via vasculogenesis only occurs during embryogenesis, and that angiogenesis only occurs in the adult organism has been challenged recently (Asahara and Isner, 2002; Carmeliet, 2003; Luttun et al., 2002; Rafii et al., 2002). Angiogenesis is generally suppressed in healthy adult organisms and is turned on temporarily in settings such as the female reproduction cycle or during tissue repair processes (Folkman and Shing, 1992). The "*angiogenic switch*" (Augustin, 2003; Carmeliet, 2003; Carmeliet and Jain, 2000; Folkman and Kalluri, 2003) is determined by the opposing faces of pro-angiogenic and anti-angiogenic factors (**Figure 1**). Depending on the activity on each end of the balance, the "*angiogenic switch*" is turned "*Off*", "*On*", or is in a balance. The "*angiogenic switch*" is likely turned "*On*" in several diseases such as psoriasis, rheumatoid arthritis, diabetic retinopathy, and cancer (Augustin, 2003; Carmeliet, 2003; Carmeliet and Jain, 2000; Folkman and Kalluri, 2003). Switching to an angiogenic phenotype likely requires both up-regulation of angiogenesis activators and down-regulation of angiogenesis inhibitors (Folkman, 1995; Folkman and Shing, 1992; Nyberg et al., 2005). The exact molecular mechanisms controlling the "*angiogenic switch*" are not completely understood, and so far, more than 20 stimulators as well as inhibitors of angiogenesis have been identified (**Figure 1**). Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are the most extensively studied angiogenesis inducers (Carmeliet, 2003; Carmeliet and Jain, 2000; Mesri et al., 2001). Among the various endogenous inhibitors of angiogenesis (Nyberg et al., 2005; O'Reilly et al., 1997; O'Reilly et al., 1994) certain factors like Arresten (Colorado et al., 2000), Canstatin (Kamphaus et al., 2000) and Tumstatin (Hamano and Kalluri, 2005; Maeshima et al., 2001; Maeshima et al., 2002) are derived from the extracellular matrix (ECM). The ECM is a complex structure composed of many different glycoproteins, proteoglycans and hyaluronic acid. All basement membranes – a specialized form of extracellular matrix - are composed of type IV collagen, laminins, entactins, elastin, fibrillin, fibrin, and fibronectins (Hudson et al., 2003; Kalluri et al., 1997; Timpl, 1996). Six different type IV collagen α chains (α1-6) have been identified (Hostikka et al., 1990; Hostikka and Tryggvason, 1988; Kalluri, 2003; Leinonen et al., 1994; Maeshima et al., 2001; Mariyama et al., 1994; Myers et al., 1990; Prockop and Kivirikko, 1995; Soininen et al., 1987; Zhou et al., 1994) which share a basic domain configuration of a N-terminal 7S domain, a middle triple helical domain with its characteristic Gly-X-Y motif, and at the C-terminus a globular non-collagenous (NC1) domain, respectively (Kalluri, 2003; Timpl et al., 1981). Via the NC1 domains, the α-chains possess the ability for self-assembly into triple helices to form a network of three-dimensional type IV collagen suprastructure (Herbst et al., 1988; Khoshnoodi et al., 2006; Koliakos et al., 1989; Paulsson, 1992; Tsilibary et al., 1988; Tsilibary et al., 1990). This type IV collagen suprastructure

serves many functions, such as providing structural support and an anchorage for cells, and also serving as ligands for cell surface receptors (Colorado et al., 2000; Kalluri, 2002; Kamphaus et al., 2000; Maeshima et al., 2000a; Maeshima et al., 2000b). Moreover, type IV collagen has been shown to have a crucial role in endothelial cell proliferation and cell behavior (Madri, 1997). Inhibition of collagen metabolism has been demonstrated anti-angiogenic features (Maragoudakis et al., 1993). These experiments further substantiated the speculation that blood vessel formation and survival are indispensably connected with proper collagen synthesis and deposition in basement membranes (Maragoudakis et al., 1993). Furthermore, the NC1 domains of several type IV collagen α chains have been shown as novel integrin ligands and inhibitors of angiogenesis and tumor growth (Colorado et al., 2000; Kamphaus et al., 2000; Kawaguchi et al., 2006; Maeshima et al., 2000a; Maeshima et al., 2000b; Maeshima et al., 2001; Maeshima et al., 2002; Magnon et al., 2005; Panka and Mier, 2003; Petitclerc et al., 2000; Sudhakar et al., 2005).

Arresten

Arresten is the 26 kDa C-terminal globular non-collagenous (NC1) domain of the α1 chain of type IV collagen (α1[IV]NC1) (Colorado et al., 2000). It was initially isolated from human placental basement membrane, and human Arresten was recombinantly produced in *E. coli* and 293 human embryonic kidney cells (Colorado et al., 2000). Arresten inhibits the proliferation of basic fibroblast growth factor (bFGF)-stimulated human endothelial cells in a dose dependent manner and induces apoptosis. Arresten also inhibits endothelial cell migration and tube formation, yet has no effect on many non-endothelial cells. In Matrigel plug angiogenesis assays, neo-vascularization is significantly inhibited by Arresten. Arresten inhibits the growth of human xenograft tumors in nude mice with a marked decrease of CD31 positive tumor vasculature. Furthermore, Arresten inhibits the development of metastasis. The anti-angiogenic effect of Arresten most likely is mediated via binding to α1β1 integrin and via inhibition of MAPK signaling in endothelial cells (Colorado et al., 2000; Sudhakar et al., 2005).

Canstatin

Canstatin is the 24 kDa NC1 domain of the α2 chain of type IV collagen (α2[IV]NC1), and human Canstatin was recombinantly produced in *E. coli* and 293 human embryonic kidney cells (Kamphaus et al., 2000). Canstatin inhibits the proliferation of fetal calf serum (FCS)-stimulated human endothelial cells in a dose dependent manner and induces apoptosis, and it has no effect on non-endothelial cells. Canstatin also inhibits endothelial cell migration and tube formation (He et al., 2004; He et al., 2003; Hou et al., 2004; Kamphaus et al., 2000). It has been shown with human umbilical vein endothelial cells (HUVEC), that Canstatin inhibits the phosphorylation of Akt, focal adhesion kinase (FAK), mammalian target of rapamycin (mTOR), eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), and ribosomal S6 kinase. Canstatin induces Fas ligand (FasL) expression and Fas dependent apoptosis (Panka and Mier, 2003), and it activates procaspase-8 and -9 cleavage (Panka and Mier, 2003). Recently the functional receptor for canstatin was proposed as the αvβ3 and αvβ5 integrins (Magnon et al., 2005).

Tumstatin

Among the endogenous angiogenesis inhibitors derived from type IV collagen, Tumstatin has been studied most extensively. Tumstatin was identified as the 28 kDa NC1 domain of the α3 chain of type IV collagen (α3[IV]NC1) (Maeshima et al., 2000a; Maeshima et al., 2000b). Tumstatin was purified from MMP degraded basement membrane preparations from the kidney, placenta and testis. Tumstatin inhibits neo-vascularization in Matrigel plug assays and suppresses tumor growth in many different mouse cancer models (Maeshima et al., 2000a; Maeshima et al., 2000b; Maeshima et al., 2001; Maeshima et al., 2002). Furthermore, Tumstatin inhibits bFGF stimulated HUVEC proliferation and induces apoptosis in a dose dependent manner (Maeshima et al., 2000a; Maeshima et al., 2000b). In type IV collagen α3 chain knockout ($COL4A3^{-/-}$) mice which are also deficient in Tumstatin, an increased pathological angiogenesis and accelerated tumor growth is observed. This effect can be reversed if exogenous Tumstatin is administered to the mice at physiologic circulating concentration (Hamano et al., 2003). Tumstatin also binds and inhibits the proliferation of melanoma cells (Han et al., 1997). The specific amino acid sequence SNS (189-191) is required for adhesion and inhibition of proliferation of melanoma cells (Han et al., 1997), but this region is not responsible for the anti-angiogenic activity of Tumstatin (Maeshima et al., 2000b). Tumstatin binds to endothelial cells via αvβ3 integrin (Maeshima et al., 2000a; Maeshima et al., 2000b; Maeshima et al., 2001; Maeshima et al., 2002; Petitclerc et al., 2000), and the αvβ3 integrin binding is facilitated by an RGD-independent mechanism (Maeshima et al., 2000a). Deletion mutagenesis reveals that αvβ3 integrin binding is necessary for the anti-angiogenic activity of Tumstatin, and this activity is restricted to amino-acids 54-132 (Tum-5) within the 244 amino acids of full-length recombinant Tumstatin (Maeshima et al., 2001). The anti-angiogenic site of Tumstatin was further localized to a peptide of 25 amino-acids (T7-peptide) consisting of the residues 74-98 (Maeshima et al., 2001). The binding of Tumstatin to αvβ3 integrin inhibits CAP-depending protein translation via downregulation of mTOR in the proliferating endothelial cells (Maeshima et al., 2000a; Maeshima et al., 2000b; Maeshima et al., 2001; Maeshima et al., 2002). Binding of Tumstatin to αvβ3 integrin furthermore directly inhibits tumor growth and is dependent on an intact PTEN/Akt pathway (Kawaguchi et al., 2006). Most recently, the activity of Tumstatin has been shown to be mediated by a p53-mediated up-regulation of α2(II) collagen prolyl-4-hydroxylase (Folkman, 2006; Teodoro et al., 2006).

Summary

In the last three decades it has become clear that angiogenesis is a complex and highly regulated process. Many pro- and anti-angiogenic factors have been identified during this time period, which are referred to as the "*angiogenic cocktail*" (Augustin, 2003). Among these angiogenic molecules several endogenous angiogenesis inhibitors have been discovered. Some of them are novel degradation protein fragments of type IV collagen in the basement membrane, namely Arresten (α1[IV]NC1) (Colorado et al., 2000), Canstatin (α2[IV]NC1) (Kamphaus et al., 2000), and Tumstatin (α3[IV]NC1) (Maeshima et al., 2000a; Maeshima et al., 2000b). In angiogenic diseases like diabetic retinopathy or cancer, the "*angiogenic switch*" is turned "*ON*" due to up-regulation of pro-angiogenic factors in

comparison with angiogenesis inhibitors. But what causes such an angiogenic switch still remains unclear. Nevertheless, anti-angiogenesis research offers a clinical application in cancer and other diseases (Brooks et al., 1994; Carmeliet and Jain, 2000; Folkman, 1995; Jain and Carmeliet, 2001; Jimenez et al., 2000; Maione et al., 1990). To restore the balance between pro- and anti-angiogenic factors or to inhibit pro-angiogenic action, (i) angiogenesis inhibitors can be administered (Folkman, 2007), or (ii) endogenous angiogenesis inhibitors can be induced to accumulate from their storage pools in the extracellular matrix. Future research will shed further light on the precise function of these inhibitors in regulating angiogenesis in health and disease.

Acknowledgments

This work was partially supported by grants from the US National Institutes of Health DK55001 (R.K.), DK62987 (R.K.), DK61688 (R.K.), AA53194 (R.K.), and a research fund from the Division of Matrix Biology at Beth Israel Deaconess Medical Center. T.M.M. is supported by the German Research Foundation DFG (MU 2298/2-2).

References

Asahara T, Isner JM. Endothelial progenitor cells for vascular regeneration. J Hematother Stem Cell Res. 2002; 11:171–8. [PubMed: 11983091]

Augustin HG. [Angiogenesis research--quo vadis?]. Ophthalmologe. 2003; 100:104–10. [PubMed: 12589453]

Brooks PC, et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell. 1994; 79:1157–64. [PubMed: 7528107]

Carmeliet P. Angiogenesis in health and disease. Nat Med. 2003; 9:653–60. [PubMed: 12778163]

Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000; 407:249–57. [PubMed: 11001068]

Colorado PC, et al. Anti-angiogenic cues from vascular basement membrane collagen. Cancer Res. 2000; 60:2520–6. [PubMed: 10811134]

Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. Ann Surg. 1972; 175:409–16. [PubMed: 5077799]

Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995; 1:27–31. [PubMed: 7584949]

Folkman J. Tumor suppression by p53 is mediated in part by the antiangiogenic activity of endostatin and tumstatin. Sci STKE. 2006:pe35. 2006. [PubMed: 17003465]

Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov. 2007; 6:273–86. [PubMed: 17396134]

Folkman, J.; Kalluri, R. Tumor Angiogenesis. PC Decker Inc.; Hamilton, Ontario, Canada: 2003.

Folkman J, Shing Y. Angiogenesis. J Biol Chem. 1992; 267:10931–4. [PubMed: 1375931]

Hamano Y, Kalluri R. Tumstatin, the NC1 domain of alpha3 chain of type IV collagen, is an endogenous inhibitor of pathological angiogenesis and suppresses tumor growth. Biochem Biophys Res Commun. 2005; 333:292–8. [PubMed: 15979458]

Hamano Y, et al. Physiological levels of tumstatin, a fragment of collagen IV alpha3 chain, are generated by MMP-9 proteolysis and suppress angiogenesis via alphaV beta3 integrin. Cancer Cell. 2003; 3:589–601. [PubMed: 12842087]

Han J, et al. A cell binding domain from the alpha3 chain of type IV collagen inhibits proliferation of melanoma cells. J Biol Chem. 1997; 272:20395–401. [PubMed: 9252346]

Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996; 86:353–64. [PubMed: 8756718]

He GA, et al. The C-terminal domain of canstatin suppresses in vivo tumor growth associated with proliferation of endothelial cells. Biochem Biophys Res Commun. 2004; 318:354–60. [PubMed: 15120609]

He GA, et al. Canstatin-N fragment inhibits in vitro endothelial cell proliferation and suppresses in vivo tumor growth. Biochem Biophys Res Commun. 2003; 312:801–5. [PubMed: 14680836]

Herbst TJ, et al. Differential effects of laminin, intact type IV collagen, and specific domains of type IV collagen on endothelial cell adhesion and migration. J Cell Biol. 1988; 106:1365–73. [PubMed: 3360855]

Hostikka SL, et al. Identification of a distinct type IV collagen alpha chain with restricted kidney distribution and assignment of its gene to the locus of X chromosome-linked Alport syndrome. Proc Natl Acad Sci U S A. 1990; 87:1606–10. [PubMed: 1689491]

Hostikka SL, Tryggvason K. The complete primary structure of the alpha 2 chain of human type IV collagen and comparison with the alpha 1(IV) chain. J Biol Chem. 1988; 263:19488–93. [PubMed: 3198637]

Hou WH, et al. Recombinant mouse canstatin inhibits chicken embryo chorioallantoic membrane angiogenesis and endothelial cell proliferation. Acta Biochim Biophys Sin (Shanghai). 2004; 36:845–50. [PubMed: 15592653]

Hudson BG, et al. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med. 2003; 348:2543–56. [PubMed: 12815141]

Jain RK, Carmeliet PF. Vessels of death or life. Sci Am. 2001; 285:38–45. [PubMed: 11759584]

Jimenez B, et al. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. Nat Med. 2000; 6:41–8. [PubMed: 10613822]

Kalluri R. Discovery of type IV collagen non-collagenous domains as novel integrin ligands and endogenous inhibitors of angiogenesis. Cold Spring Harb Symp Quant Biol. 2002; 67:255–66. [PubMed: 12858548]

Kalluri R. Basement membranes: structure, assembly and role in tumour angiogenesis. Nat Rev Cancer. 2003; 3:422–33. [PubMed: 12778132]

Kalluri R, et al. Isoform switching of type IV collagen is developmentally arrested in X- linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. J Clin Invest. 1997; 99:2470–8. [PubMed: 9153291]

Kamphaus GD, et al. Canstatin, a novel matrix-derived inhibitor of angiogenesis and tumor growth. J Biol Chem. 2000; 275:1209–15. [PubMed: 10625665]

Kawaguchi T, et al. The PTEN/Akt pathway dictates the direct alphaVbeta3-dependent growth-inhibitory action of an active fragment of tumstatin in glioma cells in vitro and in vivo. Cancer Res. 2006; 66:11331–40. [PubMed: 17145879]

Khoshnoodi J, et al. Molecular recognition in the assembly of collagens: terminal noncollagenous domains are key recognition modules in the formation of triple helical protomers. J Biol Chem. 2006; 281:38117–21. [PubMed: 17082192]

Koliakos GG, et al. The binding of heparin to type IV collagen: domain specificity with identification of peptide sequences from the alpha 1(IV) and alpha 2(IV) which preferentially bind heparin. J Biol Chem. 1989; 264:2313–23. [PubMed: 2914908]

Leinonen A, et al. Complete primary structure of the human type IV collagen alpha 4(IV) chain. Comparison with structure and expression of the other alpha (IV) chains. J Biol Chem. 1994; 269:26172–7. [PubMed: 7523402]

Luttun A, et al. Placental growth factor (PlGF) and its receptor Flt-1 (VEGFR-1): novel therapeutic targets for angiogenic disorders. Ann N Y Acad Sci. 2002; 979:80–93. [PubMed: 12543719]

Madri JA. Extracellular matrix modulation of vascular cell behaviour. Transpl Immunol. 1997; 5:179–83. [PubMed: 9402683]

Maeshima Y, et al. Two RGD-independent alpha vbeta 3 integrin binding sites on tumstatin regulate distinct anti-tumor properties. J Biol Chem. 2000a; 275:23745–50. [PubMed: 10837460]

Maeshima Y, et al. Distinct antitumor properties of a type IV collagen domain derived from basement membrane. J Biol Chem. 2000b; 275:21340–8. [PubMed: 10766752]

Maeshima Y, et al. Identification of the anti-angiogenic site within vascular basement membrane-derived tumstatin. J Biol Chem. 2001; 276:15240–8. [PubMed: 11278365]

Maeshima Y, et al. Tumstatin, an endothelial cell-specific inhibitor of protein synthesis. Science. 2002; 295:140–3. [PubMed: 11778052]

Magnon C, et al. Canstatin acts on endothelial and tumor cells via mitochondrial damage initiated through interaction with alphavbeta3 and alphavbeta5 integrins. Cancer Res. 2005; 65:4353–61. [PubMed: 15899827]

Maione TE, et al. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science. 1990; 247:77–9. [PubMed: 1688470]

Maragoudakis ME, et al. Basement membrane biosynthesis as a target for developing inhibitors of angiogenesis with anti-tumor properties. Kidney Int. 1993; 43:147–50. [PubMed: 7679456]

Mariyama M, et al. Complete primary structure of the human alpha 3(IV) collagen chain. Coexpression of the alpha 3(IV) and alpha 4(IV) collagen chains in human tissues. J Biol Chem. 1994; 269:23013–7. [PubMed: 8083201]

Mesri M, et al. Suppression of vascular endothelial growth factor-mediated endothelial cell protection by survivin targeting. Am J Pathol. 2001; 158:1757–65. [PubMed: 11337373]

Myers JC, et al. Molecular cloning of alpha 5(IV) collagen and assignment of the gene to the region of the X chromosome containing the Alport syndrome locus. Am J Hum Genet. 1990; 46:1024–33. [PubMed: 2339699]

Nyberg P, et al. Endogenous inhibitors of angiogenesis. Cancer Res. 2005; 65:3967–79. [PubMed: 15899784]

O'Reilly MS, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell. 1997; 88:277–85. [PubMed: 9008168]

O'Reilly MS, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell. 1994; 79:315–28. [PubMed: 7525077]

Panka DJ, Mier JW. Canstatin inhibits Akt activation and induces Fas-dependent apoptosis in endothelial cells. J Biol Chem. 2003; 278:37632–6. [PubMed: 12876280]

Paulsson M. Basement membrane proteins: structure, assembly, and cellular interactions. Crit Rev Biochem Mol Biol. 1992; 27:93–127. [PubMed: 1309319]

Petitclerc E, et al. New functions for non-collagenous domains of human collagen type IV. Novel integrin ligands inhibiting angiogenesis and tumor growth in vivo. J Biol Chem. 2000; 275:8051–61. [PubMed: 10713126]

Prockop DJ, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem. 1995; 64:403–34. [PubMed: 7574488]

Rafii S, et al. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? Nat Rev Cancer. 2002; 2:826–35. [PubMed: 12415253]

Sauer G, Deissler H. Angiogenesis: prognostic and therapeutic implications in gynecologic and breast malignancies. Curr Opin Obstet Gynecol. 2003; 15:45–9. [PubMed: 12544501]

Soininen R, et al. Complete primary structure of the alpha 1-chain of human basement membrane (type IV) collagen. FEBS Lett. 1987; 225:188–94. [PubMed: 3691802]

Sudhakar A, et al. Human alpha1 type IV collagen NC1 domain exhibits distinct antiangiogenic activity mediated by alpha1beta1 integrin. J Clin Invest. 2005; 115:2801–10. [PubMed: 16151532]

Teodoro JG, et al. p53-mediated inhibition of angiogenesis through up-regulation of a collagen prolyl hydroxylase. Science. 2006; 313:968–71. [PubMed: 16917063]

Timpl R. Macromolecular organization of basement membranes. Curr Opin Cell Biol. 1996; 8:618–24. [PubMed: 8939648]

Timpl R, et al. A network model for the organization of type IV collagen molecules in basement membranes. Eur J Biochem. 1981; 120:203–11. [PubMed: 6274634]

Tsilibary EC, et al. Heparin type IV collagen interactions: equilibrium binding and inhibition of type IV collagen self-assembly. J Biol Chem. 1988; 263:19112–8. [PubMed: 3198614]

Tsilibary EC, et al. Identification of a multifunctional, cell-binding peptide sequence from the a1(NC1) of type IV collagen. J Cell Biol. 1990; 111:1583–91. [PubMed: 2211826]

Zhou J, et al. Complete primary structure of the sixth chain of human basement membrane collagen, alpha 6(IV). Isolation of the cDNAs for alpha 6(IV) and comparison with five other type IV collagen chains. J Biol Chem. 1994; 269:13193–9. [PubMed: 8175748]

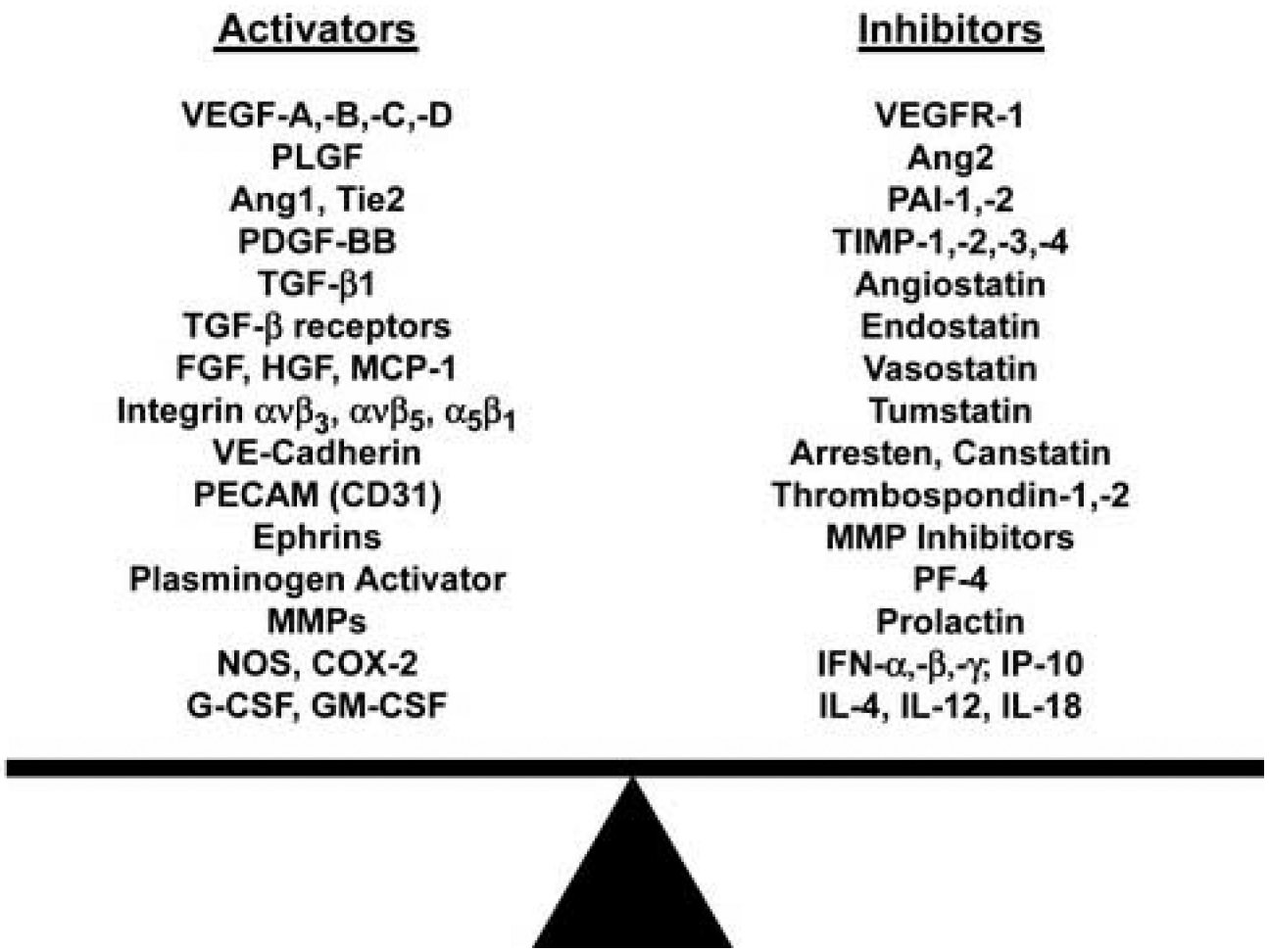

Figure 1.

Activators and inhibitors of angiogenesis. More than 20 angiogenesis stimulators and inhibitors are known now. Among the large number of angiogenesis inhibitors, there is a subset of endogenous matrix-derived angiogenesis inhibitors, e.g. Arresten, Canstatin, Endostatin, and Tumstatin.

Table 1

Basement membrane type IV collagen derived Angiogenesis Inhibitors.

	MW	Domain	Full length precursor protein	Receptor candidate	Activity	References
Arresten	26 kDa	NC1	Type IV collagen α1 chain	α1β1 Integrin	Suppression of tumor growth Inhibition of endothelial cell proliferation and migration Induction of apoptosis	(Colorado et al., 2000)
Canstatin	24 kDa	NC1	Type IV collagen α2 chain	α3β1 Integrin αvβ3 Integrin αvβ5 Integrin	Suppression of tumor growth Inhibition of endothelial cell proliferation and migration Induction of apoptosis	(He et al., 2004; He et al., 2003; Hou et al., 2004; Kamphaus et al., 2000; Magnon et al., 2005; Panka and Mier, 2003)
Tumstatin	28 kDa	NC1	Type IV collagen α3 chain	αvβ3 Integrin αvβ1 Integrin	Suppression of tumor growth Inhibition of endothelial cell proliferation Induction of apoptosis	(Hamano et al., 2003; Kawaguchi et al., 2006; Maeshima et al., 2000a; Maeshima et al., 2000b; Maeshima et al., 2001; Maeshima et al., 2002; Petitclerc et al., 2000; Teodoro et al., 2006)