Chapter 4

A Review on Pro- and Angiogenic Factors as Targets of Clinical Intervention

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**ABSTRACT**

Angiogenesis plays an important role in physiology and pathology. It is a tightly regulated process, influenced by the microenvironment and modulated by a multitude of pro- and anti-angiogenic factors. A thorough understanding of the angiogenic process may lead to novel therapies to target ischemic vascular diseases as well as diseases characterized by excessive angiogenesis such as rheumatoid arthritis, psoriasis or tumors.

This review gives an overview of the (groups of) factors involved in different steps of the angiogenic process, divided into factors affecting endothelial proliferation and migration and factors affecting blood coagulation, fibrinolysis and the degradation of basement membranes and the extra-cellular matrix, with a specific emphasis on angiopoietins and their related growth factors. The therapeutic implications of these factors are discussed.
INTRODUCTION

ANGIOGENESIS is the outgrowth of new blood vessels from pre-existing vasculature. It is an essential process in embryogenesis and wound healing but also plays a major role in several pathologic processes such as tumor vascularization, diabetic retinopathy, psoriasis, and rheumatoid arthritis. A large number of anti-angiogenic factors are in the process of being tested in clinical trials. Pro-angiogenic therapy has seen some success throughout the last decade, namely with gene therapeutic clinical trials in critical limb ischemia and myocardial ischemia.

Angiogenic sprouting of blood vessels occurs in a series of steps, which can roughly be divided into a destabilization phase, a proliferation and migration phase, and a maturation phase. All these steps offer potential points of pro- or anti-angiogenic clinical intervention.

Vessel destabilization

In response to an angiogenic stimulus (injury, inflammation, hypoxia), endothelial cells (EC) become activated, attract and bind leukocytes and blood platelets that release a multitude of pro- and anti-angiogenic factors. The EC further loosen their contacts with each other, their basement membrane (BM) and their supporting peri-endothelial cells (pericytes and smooth muscle cells (SMC) leading to increased vascular permeability and deposition of fibrin into the extra-vascular space, vessel wall disassembly and BM degradation.

Proliferation and Migration

The activated EC migrate on and into the fibrin scaffold and invade the underlying extra-cellular matrix (ECM) towards the angiogenic stimulus and proliferate. Ultimately they form a capillary lumen by aligning.

Vessel maturation

Once a new vessel has been formed, EC proliferation and migration are inhibited and a new BM is secreted. The junctional complexes between the EC as well as with the BM mature and peri-endothelial cells are recruited and differentiate.

Yet, sprouting is not the only way to enlarge the vascular network: larger vessels can split longitudinally into two daughter vessels by intussusception. Furthermore bone-marrow-derived endothelial precursor cells have been shown to home to sites of blood vessel growth, differentiate, proliferate and form new vessels. Finally, vascularization can be augmented by enlargement of pre-existing vessels as is seen in collateral outgrowth. Still, the term angiogenesis is most widely used for the above-mentioned vascular sprouting.

The great variety of angiogenic factors and their inhibitors can be divided into factors mainly acting on EC proliferation and/or migration (Table 1) and factors that affect the BM and ECM, as a physical barrier as well as a pool of sequestered growth factors (Table 2).
The factors affecting the BM and ECM do also have a role during migration and proliferation of EC but they have important functions in the process of destabilization. As many factors modulate each other's response, this classification cannot be exhaustive.

This review focuses on vascular angiogenesis in the adult with emphasis on potentially therapeutic pro- and anti-angiogenic factors. Reviews highlighting specifically embryonic vasculogenesis and angiogenesis and lymph-angiogenesis have been published.10,11

Table 1. Factors affecting EC Proliferation and Migration

<table>
<thead>
<tr>
<th>PRO-ANGIOGENIC FACTORS</th>
<th>REF</th>
<th>INHIBITORS AND ANTI-ANGIOGENIC FACTORS</th>
<th>REF</th>
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<tbody>
<tr>
<td>RTK-binding factors</td>
<td></td>
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<tr>
<td>VEGF</td>
<td>4-7, 12, 20</td>
<td>mAbs to VEGF or VEGFRs, soluble VEGFR, RNA-aptamers, RTK-inhibitors</td>
<td>1, 2, 18, 19</td>
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<td>bFGF</td>
<td>6, 22, 26, 27</td>
<td>mAbs, Suramin, Suradistas, polysaccharides, peptides, IFNα, RTK inhibitors</td>
<td>17, 22, 23, 28</td>
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<tr>
<td>PDGF</td>
<td>3</td>
<td>RTK inhibitors (Gleevec)</td>
<td>29-32</td>
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<tr>
<td>EGF</td>
<td>3</td>
<td>mAbs, RTK inhibitors (Iressa)</td>
<td>2</td>
</tr>
<tr>
<td>Ang1, Ang2, TIE2-mab</td>
<td>46, 57, 59</td>
<td>Ang2, Gna1, soluble TIE2</td>
<td>46, 52, 86</td>
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<tr>
<td>CDT6, ARP1, -2, PGAR*</td>
<td>60, 54, 56, 64-67</td>
<td>Angioarrestin</td>
<td>76</td>
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<tr>
<td>Ephrins</td>
<td>90</td>
<td>soluble EphA2</td>
<td>90, 94</td>
</tr>
<tr>
<td>Platelet-derived</td>
<td></td>
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<tr>
<td>regulators</td>
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<tr>
<td>HGF</td>
<td>97</td>
<td>Angiostatin, HGF/NK4</td>
<td>98, 99</td>
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<td>TP</td>
<td>101</td>
<td>mAb, TPI, 2dLr</td>
<td>101, 120, 121</td>
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<tr>
<td>NPY</td>
<td>122, 123</td>
<td>PF4</td>
<td>124</td>
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</table>

* CDT6 is also known as AngX, PGAR is also known as HFARP, FIAF and ANGPTL4
FACTORS AFFECTING ENDOTHELIAL PROLIFERATION AND MIGRATION

a) The Vascular Endothelial Growth Factor (VEGF)-family and its receptors

The VEGF family and its receptors have been known for long to play a central, specific role in angiogenesis.\textsuperscript{12} VEGF and its receptors mediate vascular permeability, endothelial proliferation, migration and survival.

Table 2. Factors affecting the Basement Membrane and Extra-cellular Matrix

<table>
<thead>
<tr>
<th>PRO-ANGIOGENIC FACTORS</th>
<th>REF</th>
<th>INHIBITORS AND ANTI-ANGIOGENIC FACTORS</th>
<th>REF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation</td>
<td></td>
<td>TFPI, mAbs, LMWH</td>
<td>95, 132</td>
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<td></td>
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<td>TFPI, ATIII, TM, Hirudin</td>
<td>95, 128</td>
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<td></td>
<td></td>
<td>TSP-1, ABT-510</td>
<td>130, 131</td>
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<td></td>
<td></td>
<td>Heparin, LMWH</td>
<td>132-135</td>
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<tr>
<td>Thrombin</td>
<td>95</td>
<td></td>
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<tr>
<td>Fibrinolysis</td>
<td>129</td>
<td>PAI-1, -2, uPAR-binding peptide, Maspin, Aprotinin</td>
<td>136, 137, 145, 132</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1, -2, Angiostatin, Maspin, Aprotinin</td>
<td>139, 140, 144, 132</td>
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<tr>
<td></td>
<td></td>
<td>a2antiplasmin, a2macroglobulin</td>
<td>129</td>
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<tr>
<td>uPA</td>
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<tr>
<td>tPA</td>
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<tr>
<td>Plasmin</td>
<td>129</td>
<td></td>
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<tr>
<td>Proteolysis</td>
<td>165</td>
<td>TIMPs, Endostatin, synthetic peptides (Batimastat,Marimastat), AG3340, Minocycline</td>
<td>148, 159, 155, 1</td>
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<td>MMPs</td>
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<td></td>
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<tr>
<td>Chymases</td>
<td>146</td>
<td>BCEAB</td>
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<tr>
<td>Heparanases</td>
<td>147</td>
<td>Suramin analogues</td>
<td>147</td>
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<tr>
<td>Endogenous inhibitors</td>
<td></td>
<td>denatured Collagen mAbs</td>
<td>166</td>
</tr>
<tr>
<td>Angiostatin, Endostatin, Arresten, Canstatin, Tumatin, Restin, Prolactin, PEX, Vasostatin, Kininostatin</td>
<td>139, 161, 164, 165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integrins</td>
<td>167</td>
<td>mabs (Vitaxin) or RGD peptides (Anginex, Cilengitide)*</td>
<td>167, 168, 170</td>
</tr>
</tbody>
</table>

* Integrin avb3 and avb5 could also be antiangiogenic\textsuperscript{166}
Vascular Endothelial Growth Factor

VEGF-A (also known as vascular permeability factor VPF, commonly termed VEGF) is a 34-46 kDa secreted glycoprotein that assembles into a disulphide-linked homodimer. There are at least eight known human isoforms that are the result of alternative RNA splicing and termed VEGF$_{121}$, VEGF$_{145}$, VEGF$_{162}$, VEGF$_{165}$, VEGF$_{165B}$, VEGF$_{183}$, VEGF$_{189}$ and VEGF$_{206}$ according to their length in amino acids. VEGF$_{121}$ is soluble whereas the larger isoforms bind to the cell surface or to the ECM through heparin- or proteoglycane-binding domains. The predominant isoform in humans is VEGF$_{165}$ often termed VEGF. It binds to VEGF-receptor 1 (VEGFR1), VEGFR2, Neuropilin-1 and Neuropilin-2 on EC.

VEGF-B is approximately 44% identical to VEGF, it exists in the two isoforms VEGF-B167 and VEGF-B186 and can form heterodimers with VEGF. VEGF-B binds only to VEGFR1 inducing expression and increased activity of urokinase-type plasminogen activator (uPA, see below) and plasminogen-activator inhibitor 1 (PAI-1), suggesting a role in ECM degradation and EC migration.

VEGF-C and VEGF-D (also known as c-fos induced growth factor FIGF) form a subgroup within the VEGF-family as they consist of a central VEGF homology domain with N- and C-terminal extensions, that are cleaved during protein maturation and are not seen in the other VEGFs or placenta growth factor (PIGF). Both, VEGF-C and -D consist of non-covalent dimers and bind to VEGFR2 and VEGFR3. VEGF-C seems to play a predominant role in lymph-angiogenesis, and is mainly expressed during embryogenesis whereas VEGF-D is also expressed in adult heart, lung and skeletal muscle. VEGF-E is the protein encoded for by a gene from the parapox Orf virus.

PIGF is mainly expressed in the placenta and tumors and forms disulphide-linked homodimers but can also form heterodimers with VEGF. It exists in three splice variants that all bind to VEGFR1 but only PIGF-2 can also bind to neuropilin-1 and heparin. The bioavailability of VEGF and basic fibroblast growth factor (bFGF) and therefore their capacity to induce angiogenesis might be controlled by PIGF-2.$^{13}$ PIGF induced angiogenesis in vivo to an extent comparable to VEGF.$^{14}$

The three VEGF-receptors VEGFR1 (= Flt-1 = Fms-like tyrosine kinase1), VEGFR2 (=Flk-1 = KDR = kinase domain region) and VEGFR3 (=Flt-4) are receptors with 7 immunoglobulin-like domains in their extra-cellular part and a split tyrosine kinase domain intra-cellularly. These receptors are mostly expressed on endothelial cells.

In contrast to VEGFR1-3, neuropilins are not RTK’s. The two family members neuropilin-1 (Np1) and -2 (Np2) contain a single transmembrane domain and function as semaphorin receptors for axon guidance during embryonic development as well as co-receptors for VEGFR-1 and -2, thereby playing a role in the cardiovascular development as well as in adult angiogenesis. VEGFR-signaling is inefficient in the absence of Np1. Furthermore soluble dimers of the extra-cellular domain of Np1 promote VEGF-induced angiogenesis whereas monomers inhibit it.$^{15}$

Angiogenesis can be blocked by ablation of VEGF by monoclonal antibodies (MA) to VEGF or its receptor as well as soluble decoy receptors and small molecule inhibitors of the tyrosine kinase activity of the VEGFRs. Another approach is the generation of a mutant heterodimeric VEGF-molecule to block the receptor binding sites, inhibiting signal transduction.$^{16}$ Small molecules inhibiting the tyrosine kinase domain of VEGFR as SU5416
Diagnostic and Therapeutic Aspects

(Sugen) have been shown to effectively and selectively inhibit angiogenesis in vitro and in vivo and are in the process of clinical testing. They seem to have activity in a number of tumor types. Clinical studies with antibodies, scavengers, gene therapy or blocking molecules for VEGF or its receptors or VEGF-conjugated toxins as well combinations of the above with conventional therapy have been reported with increasing success. The same accounts for pro-angiogenic VEGF (gene) therapy.

b) The Fibroblast Growth Factor (FGF) family

Unlike the VEGF-family and despite their name, FGFs are strong mitogens for many cell types, not being restricted to vascular cells or fibroblasts. They are major growth and differentiation factors in embryonic development as well as in the adult playing a role in neuronal signaling, inflammatory processes, hematopoiesis, angiogenesis, tumor growth and invasion. FGFs are small polypeptides of 155 to 268 amino acids, most of them are constitutively secreted, with a few family members remaining intracellular and a few FGFs lacking a signal sequence, still leaving the cell through an unknown mechanism, possibly involving a carrier protein. The two most extensively studied members of the FGF family, acidic FGF (aFGF, FGF1) and basic FGF (bFGF, FGF2) both adhere to this last group. The FGF family comprises 23 members to date.

FGFs strongly bind to components of the ECM as heparan sulphate proteoglycans (HSPG) from which they can be released by heparin or during ECM-breakdown. Besides sequestration in the ECM or BM, FGFs can be protected from degradation by binding to FGF-binding proteins (FGF-BP), overexpressed in various tumors. Furthermore the ECM plays a major role in FGF-signaling as HSPG has been shown to bind to FGFRs and facilitate FGF-binding. bFGF has four known alternative splice forms all inducing proliferation, chemotaxis and urokinase type plasminogen activator activity, VEGF and VEGFR2-upregulation in EC, whereas only one isoform is known for aFGF. The angiogenic activity of bFGF might partly be mediated by up-regulation of VEGF.

The FGFs bind to surface receptors (FGFR) consisting of three extra-cellular immunoglobulin-like domains, a single transmembrane domain and an intracellular tyrosine kinase domain which dimerizes upon ligand binding and initiates the intracellular signal transduction. There are four major FGFR genes that lead to a variety of receptors of different specificities by alternative splicing. Recently two new members of the FGFR gene family have been described, FGFR5 lacking an RTK-domain and FGFR6 belonging to the major histocompatibility complex (MHC) family.

Animal studies suggested that gene therapy with bFGF could lead to more mature vessels than with VEGF. Clinical trials with recombinant FGF-4 or FGF-1 protein or gene therapy have been reported showing safety, feasibility and positive angiogenic effects. Inhibition of angiogenesis has been shown with mAbs against bFGF, suramin, suradistas and their derivatives, which bind to and complex aFGF, bFGF as well as platelet derived growth factor (PDGF) and prevent them from binding to their receptors, as well as with several sulphated polysaccharides which were shown to bind to bFGF and inhibit its dimerization as well as receptor binding. A recent report showed that peptides or antibodies binding to
residues 48-58 of bFGF could inhibit its dimerization and ensuing signaling and angiogenesis.\textsuperscript{28} Furthermore interferon α (IFNα) was shown to down-regulate bFGF, leading to promising results in juvenile hemangiomas.\textsuperscript{23} Besides inhibitors of FGFs, there are also inhibitors of FGFRs. Small molecules binding to the intracellular kinase domain, inhibiting ATP binding such as SU6668 a broad spectrum receptor tyrosine kinase inhibitor have been shown to be anti-angiogenic and induce regression of established tumors and are in the process of clinical testing (for a review of a broad variety of kinase inhibitors of different specificities to FGFs, VEGF, epidermal growth factor (EGF); see ref\textsuperscript{17}).

c) **Platelet derived growth factor (PDGF)**

Besides VEGF and FGFs, PDGF has been shown to be one of the most potent angiogenesis inducers and is licensed for the treatment of neuropathic diabetic foot ulcer.\textsuperscript{3} PDGF can be secreted as a homo- or heterodimer of an A- and/or B-chain by platelets, macrophages, EC, fibroblasts and keratinocytes. It signals through two receptors α and β, of which the β-form is predominant in fibroblasts, SMC and micro-vascular EC and only recognizes PDGF-BB. Signaling leads to cellular proliferation and migration in synergy with transforming growth factor β (TGFβ) and epidermal growth factor. Inhibitors of PDGF-induced angiogenesis include specific as well as broad spectrum RTK inhibitors (SU6668\textsuperscript{29}), of which imatinib mesylate (Gleevec) is approved for the treatment of myeloid leukemia and gastro-intestinal stroma tumors and is in the phase of clinical trial for other malignancies.\textsuperscript{30} Newer RTK-inhibitors show promising results in preclinical settings.\textsuperscript{31,32}

d) **Epidermal Growth Factor (EGF)**

EGF is secreted by platelets, macrophages and monocytes and in salivary, lacrimal and duodenal glands as well as in the kidney. It has no direct effects on vascular endothelium but is nevertheless involved in tumor proliferation, metastasis, apoptosis, angiogenesis and wound healing. Its structure is similar to TGFα with which it shares a common receptor.\textsuperscript{3} Inhibitors of EGFR include mAbs (as Erbitux), small molecule inhibitors (as Iressa) and have shown promising results in clinical trials.\textsuperscript{2}

e) **Angiopoietins, related factors and TIE-receptors**

*Angiopoietins*

Since their first discovery in 1996, Angiopoietins have been a focus of growing interest in angiogenesis-research. Angiopoietin 1 (Ang1)\textsuperscript{33} was discovered as a ligand to the previously orphan TIE2 (tyrosine kinase with immunoglobulin-like loop and EGF homology domains) receptor expressed exclusively on EC. Ang2 is a natural antagonist of Ang1 with similar binding affinity to TIE2 but not inducing receptor phosphorylation.\textsuperscript{34}
Angiopoietins consist of a N-terminal signal sequence, followed by a 50 amino acid stretch, termed N domain and a coiled coil-domain that both account for multimerization and a C-terminal fibrinogen-like domain, containing six signature cysteines, that is responsible for binding and activation of the TIE2 receptor. The differences in TIE2 receptor activation between Ang1 and Ang2 have been shown to reside within the fibrinogen-like domain. Ang2 is only expressed in the ovary, placenta and uterus, organs with constant blood vessel growth and regression, as well as in (chicken) testicular development and regression and in rheumatoid arthritis synovial fibroblasts.

Ang2 expression can be up-regulated by VEGF, bFGF and hypoxia and can be down-regulated by Ang1 and TGFβ and in an autocrine way by itself.

Ang1 is secreted and binds to the ECM through the linker region between the fibrinogen-like and the coiled-coil domain in such a way that it is inaccessible for TIE2-binding, whereas Ang2 does not bind to the ECM. Upon attachment of EC, Ang1 is released from the ECM stores and binds to endothelial TIE2.

Besides enhancing EC-migration on fibronectin and collagen in a TIE2-dependent way (an effect that can be ablated by Ang2), Ang1 can also induce EC adhesion, spreading, focal contact formation and migration in a TIE2-independent manner through integrins as well as rescue EC from growth factor-deprivation induced apoptosis.

Neither Ang1 nor Ang2 alone could trigger an angiogenic response alone, but could enhance VEGF-induced angiogenesis, leading to the notion of an angiogenic balance: Ang1 signaling via TIE2 leads to vessel maturation and quiescence whereas Ang2 blocks the Ang1/TIE2 signal and leads to angiogenesis or vessel regression and apoptosis, depending on the presence of VEGF. Later evidence supported this notion.

Ang1 inhibits the activating effects of VEGF and tumor necrosis factor α (TNF α) on EC such as induction of tissue factor expression (TF) and the up-regulation of the adhesion molecules ICAM-1, VCAM-1 and E-selectin, thereby leading to decreased blood coagulation and decreased inflammatory leukocyte adhesion to EC in vitro and in vivo.

Three alternative splice variants of Ang1 were described of which only the full-length variant is able to induce TIE2 receptor phosphorylation, the other splice variants binding to TIE2 or the full-length variant thereby possibly modulating the response. There are also three alternative splice forms of Ang2, binding to TIE2, inhibiting the binding of Ang1 or full length Ang2. In addition to the alternative splice forms there are also three polymorphisms for Ang2, but no polymorphisms could be found for Ang1.

Murine Ang3 is a widely expressed, context-dependent antagonist, homologue to human Ang4, which is a TIE2 agonist, predominantly expressed in the lung. Both are not to be confused with two different reported human Ang3, one of which was later renamed to angiopoietin-related protein 1 (ARP1).

Besides the four known angiopoietins and their alternative splice forms, there are also an increasing number of angiopoietin-related factors, described below.

In rabbit ischemic hindlimbs, injection of naked plasmid encoding Ang1 but not Ang2 lead to angiogenesis. This observation underscores the importance of the synergism between
Vascular Endothelial Growth Factor

various angiogenic factors in the cascade. While Ang1 alone is unable to trigger a response,44 the anoxia induced VEGF response permits the expression of the effects of the transfected Ang1 plasmide. This could be of interest to improve human VEGF gene therapy of ischemic limbs, that was shown to lead to leaky, immature vessels,58 but no clinical trials have been reported to date. An agonist mAb to TIE2 might also hold a therapeutic promise as it was shown to induce effects reminiscent of effects as Ang1 in vitro.59

Angiopoietin related growth factors

Besides reporting the identification of Ang3 and Ang4 Valenzuela et al53 also reported the identification of two distantly related sequences termed AngX and AngY, that do appear to be no true angiopoietins, because they lack the ability to bind to TIE2 and miss one, respectively two of the signature cysteines in the fibrinogen-like domain. Their AngX had been previously described by Peek et al60 and termed cornea-derived transcript 6 (CDT6). CDT6 expression could only be detected in the cornea, where it was restricted to the stromal layer. A subsequent report about CDT6 showed a substantial murine tumor growth inhibition through deposition of large amounts of ECM by the gene product.61 The authors could not clearly relate this effect to anti-angiogenesis and concluded CDT6 to be a morphogen for the cornea [61]. In contrast, however a recent conference abstract claimed a strongly pro-angiogenic activity of CDT6 in several in vitro models and increased tumor growth in vivo.62 In another study, the gene product failed to show a pro- or anti-angiogenic effect on EC proliferation as well as in an immune-competent murine tumor model.63

According to the criterion of the 6 cysteine motif in the fibrinogen-like domain, the Ang3 of Kwak et al [54] is not to be considered as an angiopoietin as it lacks two of the 6 signature cysteines (at positions 435 and 437 of Ang1), however it is also different from AngY,53 lacking the same two cysteines. In a later report, the authors renamed their Ang3 to ARP1, describing the identification of a similar factor termed ARP2.56 In the human adult ARP1 and ARP2 are most abundant in the heart, small intestine, spleen and stomach. In the rat embryo expression was most abundant in blood vessels and skeletal muscles. ARP1 and 2 are expressed in EC and SMC and were shown to induce EC sprouting in vitro while not being mitogenic to EC.56 Kim et al also identified the hepatic fibrinogen/angiopoietin-related protein (HFARP),64 also identified as PGAR (peroxisome proliferator-activated receptor ?, angiopoietin-related),65 FIAF (fasting-induced adipose factor)66 or termed angiopoietin-like 4 (ANGPTL4).67 PGAR is an endothelial survival factor as well as a target for the transcriptional activators PPARγ and PPARα, which are associated with lipid storage, utilization and energy production. The expression of PGAR in human and murine embryos and adults is predominantly localized to liver hepatocytes, adipose tissues and placenta. Strong up-regulation is seen during periods of fasting in plasma, white adipose tissue and liver as well as in genetic models of obesity, whereas down-regulation is seen with chronic high fat feeding. The function of PGAR in angiogenesis is controversial: it was shown to be silenced in human gastric cancer, along with the anti-angiogenic gene thrombomodulin.68 On the other hand, it is up-regulated in cardiomyocytes in response to hypoxia as well as in critical limb ischemia and tumors presumably through hypoxia inducible factor 1 (HIF-1) and was shown to be pro-
angiogenic in the chick chorioallantoic membrane (CAM) assay, even in the presence of a VEGF-inhibitor.\textsuperscript{69,70}

ANGPTL3 (angiopoietin-like factor 3)\textsuperscript{71} and FARP (fibrinogen/angiopoietin related protein)\textsuperscript{72} are both closely related to PGAR, the latter only differing from it in 18 amino acids. ANGPTL3 is a liver-specific, secreted factor, binding to Integrin \(\alpha_v\beta_3\), (see Integrins) inducing haptotactic EC adhesion and migration. In vivo, ANGPTL3 increases plasma lipid levels in mice through decreased clearance due to lipoprotein lipase inhibition,\textsuperscript{73} an effect that was also seen with PGAR\textsuperscript{67} and shows angiogenic effects comparable to those of VEGF-A.\textsuperscript{74} There are no data as to the effects on angiogenesis or lipid metabolism of ANGPTL5, a secreted protein mainly expressed in the adult heart as well as in the fetal brain.\textsuperscript{75}

Angioarrestin, which is down-regulated in many tumor tissues, inhibits VEGF- and bFGF-induced EC proliferation, migration, tube formation and adhesion to the ECM as well as reduces metastasis and tumor growth in vivo.\textsuperscript{76}

**TIE2 and TIE1**

The orphan TIE1 receptor is essential in embryonic angiogenesis, counteracting the endothelial motility effects of co-expressed TIE2, inhibiting intussusceptive angiogenesis as was shown in knockout studies.\textsuperscript{77} It furthermore has a function in vascular polarity in combination with Ang1.\textsuperscript{78} Its extra-cellular domain is cleaved and shed into the blood stream upon EC activation by TNFa and VEGF\textsuperscript{79,80} as is also the case with TIE2\textsuperscript{81} and the intracellular domain has been shown to exist as a pre-formed complex with TIE2 in EC suggesting a function in modulating TIE2-signalling.\textsuperscript{82} However TIE1 has been shown to potentially inhibit endothelial apoptosis, independently from TIE2,\textsuperscript{83} the same endothelial survival effect had previously been described for Ang1-signalling through TIE2.\textsuperscript{84,85}

Anti-angiogenic gene therapy with an adeno virus encoding a soluble TIE2 receptor, blocking TIE2-signalling lead to inhibited tumor growth and metastasis,\textsuperscript{86} just as ablation of TIE2 expression in cultured EC by antisense oligonucleotides lead to endothelial apoptosis.\textsuperscript{87} Crystal structure resolution of TIE2 has shown that the receptor is self-inhibiting\textsuperscript{88} and that deletion of its carboxyl-terminus leads to enhanced kinase activity and signalling.\textsuperscript{89}

**f) Eph receptors and Ephrins**

Another group of RTKs and their ligands, playing a major role in angiogenesis are the Eph receptors and their ligands the ephrins.\textsuperscript{90} The main difference between the ephrins, VEGF, FGFs and the angiopoietins is that the ephrins ligands are not soluble but membrane-tethered. Another difference is that Eph-receptor-ephrin-binding leads to signal transduction in both cells. Furthermore the expression of ephrins and their receptors is not restricted to the endothelium as is the case with VEGFRs or TIE2.

The ephrins ligands are divided into two groups according to their attachment to the membrane: Ephrin-A (ephrin A1 - A5) are attached to the outer leaflet of the plasma membrane via a gycosilylphosphotidylinositol (GPI) anchor, whereas ephrin-B (ephrin-B1 - B3) contain a transmembrane and a cytoplasmic domain. The cytoplasmic and transmembrane
domains of class B ephrins are similar to cell surface receptor molecules. Even though class A ephrins lack transmembrane and intracellular domains, Eph receptor binding still leads to bi-directional signal transduction. This effect is attributed to adapter molecules adjacent to the ephrin in a lipid microdomain or raft. After binding and signal transduction, the receptor-ligand complex can be endocytosed or cleaved off the cell surface.

There are 14 Eph receptors divided into two groups according to their preferential ligand binding: A receptors (EphA1 - A8) and B receptors (EphB1 - EphB6). Receptor-ligand binding is promiscuous within the group and there are also examples of A-type ephrins binding to EphB receptors and vice versa. Ephrin binding to Eph receptors leads to receptor dimerization and phosphorylation of the cytoplasmic domains. The extent of receptor activation depends on ligand-multidimerization, soluble ephrins can even act as antagonists, whereas artificial clustering enables them to activate their receptors.

Eph receptors and ephrins have long been known as markers of neuronal growth, however the report that they are also specific markers of arterial or venous endothelium from the early stage of capillary plexus formation on, propelled them into the world of angiogenesis factors.

Ephrins are not mitogenic but can modulate the response to other growth factors. Their expression can be induced by TNFα and VEGF. They do not promote proliferation but migration, repulsion, adhesion and attachment to the ECM via Integrins. These effects could also act on tumor cells, as over-expression of EphA2 rendered breast epithelial cells tumourigenic. Ephrins and Eph receptors also seem to play a role in tumor angiogenesis, as Ephrin A1 and EphA2 are expressed in the vasculature of a tumor variety. A soluble EphA2-Fc receptor fusion protein could inhibit EC migration, corneal angiogenesis as well as tumor angiogenesis to the same extent as VEGF signaling in vivo, yet no clinical trial have been reported to date.

Besides the already mentioned growth factors VEGF, bFGF, PDGF, Ang1 and Ang2, platelets are a rich source of other angiogenic factors such as hepatocyte growth factor (HGF), thymidine phosphorylase (TP) and neuropeptide Y (NPY) and inhibitors as platelet factor 4 (PF4). Furthermore they contain angiogenic factors of the blood coagulation cascade as heparin, thrombin, fibrinogen, and PAI that are discussed in more detail below (reviewed in refs95,96).

g) Hepatocyte growth factor (HGF)

HGF is a neuronal survival factor, induces proliferation, migration and differentiation of various types of cells and has been shown to be a potent angiogenic factor in vitro and in vivo. The first clinical trial of HGF-gene therapy for ischemic limbs has been performed and showed some improvement in critical limb ischemia, in the absence of toxicity. Its angiogenic function seems to be mediated by the Ets family of transcription factors, ultimately leading to the expression of VEGF, MMPs and uPA and strong anti-apoptotic effects on EC. Angiostatin has been shown to inhibit HGF-signaling and angiogenesis. Furthermore proteolytic
fragments of HGF (NK4) have shown potent anti-angiogenic effects not only inhibiting HGF-induced but also VEGF- and bFGF-induced angiogenesis.

**h) Thymidine Phosphorylase (TP)**

TP was first discovered as an EC specific growth factor, abundant in human platelets. It was therefore termed platelet-derived endothelial cell growth factor (PD-ECGF, not to be confounded with PDGF). TP induces endothelial chemotaxis as well as angiogenesis in vitro and in vivo. It was also isolated from neurofibroma cells where it inhibited astrocyte proliferation and induced their differentiation and was therefore termed gliostatin (GLS). Just as bFGF, the TP enzyme lacks a signal sequence but is nevertheless secreted, although slowly, by some cell types. The angiogenic effects of TP are clearly associated with its enzymatic activity, as they could be specifically inhibited in two in vivo angiogenesis models by site-directed mutagenesis, inhibiting antibodies and inhibitors. TP catalyses the reaction of thymidine and phosphate to thymine and 2-deoxy-D-ribose-1-phosphate (2dDr1P), which can then be dephosphorylated to 2-deoxy-D-ribose (2dDr). This last compound has been identified to be crucial in the angiogenic activity of TP. 2dDr showed dose-dependent chemotactic attraction of EC similar to that of TP protein. Moreover, 2dDr but not thymidine, thymine or D-ribose were able to induce significant angiogenesis. No specific receptor for TP or 2dDr has been identified so far in mammalian cells.

In the healthy adult TP is mainly expressed in peripheral lymphocytes, platelets, lymph nodes, spleen, macrophage-like cells, stromal cells, glial cells as well as in the gastrointestinal tract, brain and bladder. A variant form of TP containing five additional N-terminal amino acids was described in human term placenta stromal cells, and found to be as angiogenic as TP. TP is expressed in a wide range of tumours, often in co-expression with VEGF, further in psoriasis, and in gastric ulcers. It is furthermore abundant in synovial fluids and serum of rheumatoid arthritis patients. TP-expression can be induced by hypoxia, VEGF, TNF, Interleukin 1α (IL-1α), IL-6, IL-8, IFNγ, ovarian steroids and xenobiotics as cyclophosphamide and taxanes. TP induces its own and matrix metalloproteinase (MMP)-expression in rheumatoid arthritis synoviocytes, as well as IL-8, VEGF and MMP expression in tumor cells. TP-transfected tumor cells showed significantly increased tumor growth in vivo. TP has been shown to induce oxidative stress, presumably by the generation of oxygen radical species by its product 2dDr1P. Hypoxia in turn, induces expression of TP and VEGF in vitro and in vivo and TP, 2dDr and VEGF are able to prevent tumor cells from ensuing apoptosis, an effect inhibited by the stereoisomer 2dLr. An inhibitor of TP function, TPI, as well as 2dLr significantly inhibited tumor growth in vivo, significantly increasing apoptosis. TP-expression in tumors has been monitored as a prognostic factor in clinical trials, as the enzyme is a target for the chemotherapeutic drugs 5-fluorouracil and methotrexate. Yet no clinical trials for either pro- or anti-angiogenesis with TP have been reported.
i) **Neuropeptide Y (NPY)**

NPY is a 36 amino-acid tyrosine-rich peptide present in all sympatthrowic nerves innervating the cardiovascular system and abundant in brain and heart. NPY is a potent mitogen for EC and SMC, inducing EC adhesion, migration and differentiation into capillaries.\(^{122}\) It was furthermore shown to induce angiogenesis in several in vivo-settings.\(^{123}\) There are five receptors with different specificities for NPY, termed Y1- Y5. Y1, Y2 and Y5 are the main receptors involved in angiogenesis. Y1 and Y2 are constitutively expressed on EC and SMC and Y5 expression in both cell types can be induced by hypoxia. Their expression is controlled by NPY and bFGF. Deletion of Y2 results in delayed wound healing in vivo.\(^{123}\) NPY is released from sympathetic nerves, platelets, immune cells and EC, this release can be stimulated by hypoxia. NPY plasma levels increase during stress, as with exposure to cold or strenuous exercise.\(^{122}\) A recent report shows that exogenous NPY induces angiogenesis in ischemic muscle, which might be of therapeutic interest.\(^{122}\)

j) **Platelet Factor 4 (PF4)**

PF4 was the first hematostatic factor shown to inhibit angiogenesis and is unique to platelets. After secretion it binds to and blocks heparin-like glycosaminoglycans on the EC surface inhibiting binding of endothelial growth factors, furthermore directly neutralizing the heparin-binding regions of growth factors. Yet there are also reports of a heparin-independent pathway of PF-4 inhibition of EC growth. It also quenches the anti-thrombotic activity of antithrombine III (ATIII) (reviewed in ref\(^{124}\)).

**FACTORS AFFECTING THE BASEMENT MEMBRANE (BM) AND EXTRA-CELLULAR MATRIX (ECM)**

a) **Blood coagulation system**

The blood coagulation system has been shown to play a major role in angiogenesis. The end product of the complex coagulation cascade is fibrin. Under physiologic conditions EC keep blood fluid by releasing anticoagulants and inhibiting blood-cell adhesion. Platelets get activated by inflammatory or tumor-secreted growth factors or components of the BM and ECM that are exposed in wounds and release growth factors into the circulation. These include VEGF, bFGF, PDGF, TP, EGF, HGF, TGFβ and Ang1. Yet platelets also release anti-angiogenic factors such as PF4, PAI-1 and TSP1 and TSP2 (thrombospondin -1 and -2) and endostatin (reviewed in ref\(^{95}\)). This growth factor release entails endothelial activation and inflammatory cell recruitment leading to release of more inflammatory cytokines and growth factors. Among the growth factors released by the activated EC is tissue factor (TF). It is a membrane glycoprotein aberrantly expressed in many tumors and has been shown to be
angiogenic. TF up-regulates VEGF on EC and is up-regulated by hypoxia as VEGF and mAbs to TF were shown to be anti-angiogenic. TF starts the coagulation cascade ultimately leading to the cleavage of the precursor prothrombin to active thrombin and two smaller prothrombin fragments (F1 and F2) that have been shown to be anti-angiogenic [126]. Thrombin is a potent platelet activator and endothelial and tumor cell mitogen, increasing metastases in vivo (reviewed in ref95). It induces endothelial Ang2-expression and secretion as well as release of VEGF and Ang1 from platelets. Thrombin terminates the coagulation cascade by cleaving soluble fibrinogen to an insoluble fibrin gel, which is the basis of blood clot formation.

TF and thrombin can both be inhibited by tissue factor pathway inhibitor (TFPI), which is expressed by EC and up-regulated in several tumor types. Thrombin can furthermore be inhibited by ATIII and hirudin which were shown to be anti-angiogenic. Another thrombin-inhibiting member of the coagulation cascade involved in angiogenesis is thrombomodulin (TM). It is found on EC and platelets and assists the normal anti-thrombotic state of resting EC, is up-regulated by VEGF and inhibits the adhesion of platelets and tumor cells to EC, thereby inhibiting metastatic spread.

Thrombospondin 1 (TSP-1) was one of the first discovered endogenous angiogenesis inhibitors. It is a major constituent of platelet α-granules, where it is complexed with activated TGF 1. Upon release it can activate EC-derived TGFα, bind fibrin, fibronectin, plasminogen, surface heparin-like glycosaminoglycans, CD36 and αvβ3 Integrins on activated EC and αIIIβ3 Integrins on activated platelets. Thrombospondins (TSP-1 and TSP-2) inhibit EC proliferation, migration and tube-formation, their over-expression leading to decreased tumor growth and vascularization in vivo, and a clinical trials with the TSP-1-mimetic ABT-510 showed promising results.

Anticoagulants are commonly used to treat tumor-associated venous thromboembolism. Yet they also harbor strong anti-angiogenic, anti-tumor effects, leading to significantly improved survival in several clinical trials.

Heparin is a broadband anticoagulant, interacting with a great number of angiogenic factors, affecting EC adhesion, migration and proliferation, inhibiting tumor growth in vitro and in vivo. Low molecular weight heparins (LMWH) have superior pharmacodynamics and specificity as well as less toxicity, leading to increased long-term survival of gynecologic malignancy patients when compared to unfractionated heparin treatment. They were shown to significantly reduce mortality alone or in combination with chemotherapy in two recent clinical trials.

b) Fibrinolysis

Insoluble fibrin can be degraded by plasmin, a broad-spectrum protease that also lyses the ECM, releasing sequestered growth factors and activates several MMPs, that also degrade the ECM. Plasmin is generated through cleavage of its inactive precursor plasminogen by either of two distinct but highly specific Proteases: tissue-type plasminogen activator (tPA) and uPA. uPA binds to its receptor uPAR, mainly expressed on EC. The two PAs can be inhibited by PA inhibitors (PAI). In platelets PAI-1 is complexed with vitronectin. PAI-1 has been shown
to be anti-angiogenic by limiting plasmin generation, just as plasmin scavengers as α₂-antiplasmin and α₂-macroglobulin (reviewed in ref^{129}). A synthetic peptide inhibiting uPA-uPAR binding has also been shown to be anti-angiogenic.^{136} Yet, the interactions of uPA and uPAR as well as PAI-1 are prerequisites of angiogenesis, the former two presumably to induce the latter to prevent (excessive) ECM proteolysis.^{137}

uPA, uPAR and PAI-1 are only expressed in angiogenic endothelium, whereas tPA is constitutively expressed in quiescent microvasculature. Both uPA and tPA can be induced by endothelial growth factors as VEGF or bFGF.^{138} The angiogenesis inhibitor angiostatin (which is a cleaved fragment of plasminogen^{139}) inhibits tPA in EC, pointing at a role of tPA in angiogenesis,^{140} further emphasized by the observation that the serine protease inhibitor aprotinin showed remarkable increases in survival time of colorectal cancer patients.^{132}

Maspin (mammary serine protease inhibitor) is a recently discovered tumor suppressor gene that is down-regulated in breast and prostate cancer, its expression inversely correlating with mutant p53 levels in tumours.^{141} It was shown to block endothelial proliferation, chemotaxis and tube formation and inhibit tumor angiogenesis.^{142} Maspin-expression lead to increased adhesion of tumor cells to the ECM.^{143,144} Maspin furthermore binds to and inhibits uPA leading to a decreased motility and invasion of tumor cells.^{145}

c) **Proteinases and their inhibitors**

The BM and ECM are a reservoir for heparin-binding growth factors such as VEGF and bFGF as well as a physical barrier conferring adhesion and stability. During angiogenesis the BM and ECM are degraded, liberating the sequestered growth factors and leaving space for EC to migrate and align into new vessels. During this phase fibrin provides a temporary scaffold that is later replaced by mature BM and ECM.

Extra-cellular proteolytic enzymes can roughly be divided into serine proteases, including the PA-plasmin system (see fibrinolysis), chymases, heparanases^{146,147} and MMPs. The MMPs in turn can be subdivided into a large group of secreted MMPs comprising collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11), other secreted MMPs (MMP-7, -12) and membrane-bound MMPs (MMP-14 = MT1-MMP, MMP-15, -16, -17, -24, -25). Secreted MMPs are secreted as pro-enzymes (zymogens) and activated extracellularly (e.g. by membrane-type MMPs), whereas membrane-type MMPs are activated intracellularly by furin-like enzymes.

Besides regulation at the expression and activation level, MMP-activity is also regulated by inhibitors as tissue inhibitors of metalloproteases (TIMP), which bind to and inhibit all activated MMPs and some zymogens, α2-macroglobulin and reck. MMP and TIMP expression is low or absent in normal tissues but is up-regulated in sites of physiologic or pathologic angiogenesis.^{148} MMPs are essential in angiogenesis as increased TIMP-expression inhibited tumour-angiogenesis,^{149,150} yet MMP activity needs to be dampened for vessel maturation, uncontrolled MMP-activity leading to regression of the neovasculature.^{151,152} Interestingly MMPs are also fibrinolytic,^{153} an activity that was only attributed to the PA-plasmin system, implying that EC can invade the temporary fibrin scaffold independently of the plasmin system.
MMPs can be inhibited by TIMPs but also by endostatin\textsuperscript{154} or synthetic inhibitors as batimastat and marimastat,\textsuperscript{155} yet although inhibition of angiogenesis has been demonstrated, MMP inhibition can also lead to increased metastasis.\textsuperscript{156,157} MMPs can act in a pro-angiogenic way by releasing ECM-bound growth factors as well as in an anti-angiogenic way by hampering vessel maturation, cleaving angiogenic factors or generating anti-angiogenic factors as angiostatin.\textsuperscript{158} Therefore their inhibitors (TIMPs or other) can also show pro- or anti-angiogenic activity, depending on the context. There are reports about angiogenesis inhibition of TIMP-1, -2 and -3 in various in vitro and in vivo settings, yet one report states pro-angiogenic activity of TIMP-1.\textsuperscript{159}

MMP-inhibitors as marimastat and AG3340 did not prove beneficial in phase III clinical trials,\textsuperscript{2} while inhibitors of chymases (as BCEAB) and heparanases (as suramin analogues) are still in a pre-clinical phase.\textsuperscript{146,147}

Besides factors degrading the BM and ECM or factors inhibiting this degradation, the inhibition of collagen synthesis has also been shown to be anti-angiogenic.\textsuperscript{160}

Angiostatin\textsuperscript{139} and endostatin\textsuperscript{161} are both endogenous inhibitors of angiogenesis, the former being a fragment of plasminogen, the latter a fragment of collagen VIII. Angiostatin has been shown to bind to ATP-synthase on the surface of EC, triggering apoptosis. It has furthermore been shown to bind to and inhibit tPA. Several MMPs as well as tumor-cell-derived plasmin thiolreductase have been shown to cleave plasmin to angiostatin.\textsuperscript{129,162} Angiostatin and endostatin have been shown to act synergistically to inhibit tumor angiogenesis.\textsuperscript{163}

The discovery that proteolytic fragments of blood coagulation/fibrinolysis system and ECM components, as angiostatin and endostatin could inhibit angiogenesis, triggered the discovery of several cleavage-derived angiogenesis regulators including arrestin, Canstatin, Tumstatin, SPARC (secreted protein, acidic, rich in Cysteine), Restin, as well as fragments of prolactin, PF4, MMP-2 (PEX) calreticulin (vasostatin), kininogen (kininostatin) and antithrombin.\textsuperscript{164,165} Fragments of HGF have also been shown to be anti-angiogenic. Yet degradation of the BM and ECM can also release cryptic pro-angiogenic domains of their components. Denatured collagen IV was shown to be angiogenic and specific mAbs could suppress angiogenesis.\textsuperscript{166}

d) Integrins

The attachment of EC to the BM, ECM and each other usually occurs in specialized focal contacts where the cytoskeletal actin filaments are linked to integrins bound to the ECM through specialized cytoplasmic proteins (e.g. talin, a-actinin). Integrins are heterodimers containing a non-covalently linked \( \alpha \) and \( \beta \) subunit. Both subunits have an extra-cellular domain binding to ECM proteins such as laminin, fibronectin, collagen and vitronectin, a single membrane-spanning region and a cytoplasmic domain. To date there are 18 known \( \alpha \)-subunits and 8 \( \beta \)-subunits assembling into a large variety of receptors, of which the \( \alpha \)VI\( \beta \)3 receptor has been most extensively studied with respect to angiogenesis. It binds to several ECM components through an RGD sequence (arginine-glycine-aspartic acid) and is almost exclusively expressed on angiogenic EC and SMC.
Inhibition of $\alpha_v\beta_3$ as well as the closely related $\alpha_v\beta_5$ by antibodies or peptide inhibitors containing an RGD-motif reduced angiogenesis and tumor growth and induced apoptosis in neovasculature.\textsuperscript{167} The $\alpha_v\beta_3$-blocker anginex also acts synergistically with chemotherapy as well as with the anti-angiogenic agent angiostatin.\textsuperscript{168} Furthermore, integrin-bound RGD-peptides are internalized, providing an opportunity to target drugs to the sites of pathological angiogenesis.\textsuperscript{169} Both, antibodies and RGD-peptides are in the process of clinical testing.\textsuperscript{1}

Besides the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, $\beta_1$ integrins as $\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_5\beta_1$ were shown to be angiogenic and antibodies against them were anti-angiogenic.\textsuperscript{167} Genetic studies have confirmed that these three $\beta_1$ integrins are essential for angiogenesis, their lack leading to embryonic death or aberrant angiogenesis. Yet interestingly, mice lacking $\alpha_v\beta_3$, $\alpha_v\beta_5$ or both are not only viable but show enhanced tumor angiogenesis pointing at an anti-angiogenic role of these Integrins, implying that "inhibitors" used so far are actually agonists. $\alpha_v\beta_3$ indeed binds and localizes anti-angiogenic factors as TSP, tumstatin, angiostatin, endostatin and PEX as well as down-regulates VEGF-C.\textsuperscript{167,170} More research is needed to clarify the pro- and anti-angiogenic mechanisms of the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ as well as the RGD peptides.

**MISCELLANEOUS FACTORS AND CONDITIONS**

As angiogenesis is such a complex and multi-factorial process, it might seem more difficult to find a factor that is not involved under any circumstances in the angiogenic process than to give a conclusive overview of all the factors involved. This section gives a glimpse of pro-and anti-angiogenic factors that do not fit into one of the above groups.

Hypoxia entails angiogenesis. One of the key players in cellular hypoxia-response is HIF-1 (hypoxia inducible factor-1). HIF-1 has been shown to act on the VEGF promoter and upregulate gene expression.\textsuperscript{171} Rapisarda et al identified small molecule inhibitors of HIF-1 that down-regulated VEGF, were topoisomerase I inhibitor analogues and associated with cyclooxygenase 2 (COX2) expression.\textsuperscript{172} The drugs LY-294002 and rapamycin inhibit HIF-1 expression by tumours,\textsuperscript{2} whereas tirapazamine (and related compounds) is a hypoxic cytokine, bioreductively activated in hypoxic cells, thereby showing anti-angiogenic as well as direct anti-tumor activity.\textsuperscript{173}

Herpes simplex virus (HSV) DNA was shown to induce angiogenesis due to large CpG motifs, which were angiogenic in themselves\textsuperscript{174} and might therefore be of gene therapeutic interest.

Tubedown-1 (TBDN-1)\textsuperscript{175} is a recently discovered acetyltransferase that is down-regulated during capillary formation in vitro, the formation and maturation of a subset of blood vessels and hematopoietic stem cells in the embryo as well as vascular remodeling in the adult ovary. It is not detected in most adult vascular beds but is abundant in adult ocular vasculature. It is down-regulated in patients with proliferative diabetic retinopathy.\textsuperscript{176} Experimental down-regulation of TBDN-1 has also been shown enhance capillary formation in vitro and to interact with TGF 2 in pruning of the vitreal vasculature.\textsuperscript{177} Recently a new
variant of TBDN-1 was described and termed TBDN-100, however the effects of this gene product on angiogenesis have yet to be reported.

Inflammation also plays a major role in pathological as well as physiological angiogenesis. Inflammatory cells as neutrophils, macrophages and lymphocytes secrete a multitude of inflammatory mediators of which many influence angiogenesis. Examples include TNFα, TGFβ, Interferon γ (IFNγ), IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF) and platelet-activating factor (PAF).

Anti-inflammatory drugs affecting the coagulation cascade as the non-steroidal anti-inflammatory drugs (NSAID) arfarin, aspirin and celecoxib have shown anti-tumor, anti-angiogenic effects through decreased VEGF-production, VEGFR2- and αVβ3-signalling.

Cytotoxic drugs do not only damage tumor cells but also the proliferating tumor vasculature. The concept of metronomic scheduling of chemotherapy has been demonstrated to yield substantial anti-angiogenic and thereby anti-tumor effects with various cytotoxic drugs.

The antibiotic fumagillin-analogue TNP-470 inhibits EC migration and proliferation, tube formation and tumor growth and metastasis by inhibiting methionine aminopeptidase II. It was shown to be effective in clinical trials alone and in combination with other factors.

Thalidomide, originally described as a sedative with severe side effects on embryogenesis has been shown to inhibit VEGF and bFGF-induced angiogenesis through a yet unknown mechanism and has shown anti-tumor activity in various phase II studies. Combrestadin A-4 is a tubule-polymerization interfering compound specifically inhibiting endothelial migration as well inducing apoptosis in proliferating EC and tumor cells, leading to extensive tumour necrosis in vivo.

Shark cartilage and its derivatives, among which AE-941 (=Neovastat) have also been shown to inhibit (bFGF-induced) angiogenesis, yet first clinical trials were disappointing. And finally, protease inhibitors (PI), commonly used in human immune-deficiency virus (HIV)-treatment were also shown to strongly inhibit VEGF and bFGF-induced angiogenesis.

**CONCLUSIONS**

Angiogenesis is a complex process involving a plethora of activating and inhibiting factors. Therapeutical intervention inducing or enhancing angiogenesis with single angiogenic factors (VEGF, bFGF) has shown some success, yet the induced neovasculature might be immature, leaky, lacking periendothelial support. Therefore the combination of angiogenic factors should be envisioned.

As many angiogenic pathways are redundant, therapeutic inhibition of angiogenesis might need an even broader approach than might be required for angiogenesis induction. Inhibitors of the intracellular tyrosine kinase moieties of growth factor receptors can block the pathways of several angiogenic factors (VEGF, FGFs, EGF), just as the antibiotic neomycin can inhibit the nuclear translocation of several angiogenic factors (angiogenin, FGFs, EGF).
The challenge lies in combining a broad anti-angiogenic action, inhibiting as many pathways as possible, without losing the specificity to pathologic angiogenesis. Many aspects of angiogenesis are still poorly understood. Yet the deciphering of the human genome and ensuing gene function studies are likely to deliver a multitude of promising new factors for pro- and anti-angiogenic therapies.
REFERENCES

Vascular Endothelial Growth Factor


Vascular Endothelial Growth Factor


Diagnostic and Therapeutic Aspects


