Thrombospondin-1 as a Paradigm for the Development of Antiangiogenic Agents Endowed with Multiple Mechanisms of Action

Marco Rusnati 1,*, Chiara Urbinati 1, Silvia Bonifacio 2, Marco Presta 1 and Giulia Taraboletti 2

1 Unit of General Pathology and Immunology, Department of Biomedical Sciences and Biotechnology, School of Medicine, University of Brescia, Brescia, 25123, Italy; E-Mails: urbinati@med.unibs.it (C.U.); presta@med.unibs.it (M.P.)
2 Tumor Angiogenesis Unit, Department of Oncology, Mario Negri Institute for Pharmacological Research, Bergamo, Italy; E-Mails: bonifacio@marionegri.it (S.B.); taraboletti@marionegri.it (G.T.)

* Author to whom correspondence should be addressed. E-Mail: rusnati@med.unibs.it; Tel.: +39-030-3717315; Fax: +39-030-3701157.

Received: 14 February 2010; in revised form: 20 April 2010 / Accepted: 22 April 2010 / Published: 23 April 2010

Abstract: Uncontrolled neovascularization occurs in several angiogenesis-dependent diseases, including cancer. Neovascularization is tightly controlled by the balance between angiogenic growth factors and antiangiogenic agents. The various natural angiogenesis inhibitors identified so far affect neovascularization by different mechanisms of action. Thrombospondin-1 (TSP-1) is a matricellular modular glycoprotein that acts as a powerful endogenous inhibitor of angiogenesis. It acts both indirectly, by sequestering angiogenic growth factors and effectors in the extracellular environment, and directly, by inducing an antiangiogenic program in endothelial cells following engagement of specific receptors including CD36, CD47, integrins and proteoglycans (all involved in angiogenesis). In view of its central, multifaceted role in angiogenesis, TSP-1 has served as a source of antiangiogenic tools, including TSP-1 fragments, synthetic peptides and peptidomimetics, gene therapy strategies, and agents that up-regulate TSP-1 expression. This review discusses TSP-1-based inhibitors of angiogenesis, their mechanisms of action and therapeutic potential, drawing our experience with angiogenic growth factor-interacting TSP-1 peptides, and the possibility of exploiting them to design novel antiangiogenic agents.
Keywords: angiogenesis; tumor; integrins; interactions; thrombospondin-1

1. Neovascularization

Angiogenesis is the process of new blood vessel formation from existing ones. It takes place in embryonic development and inflammation [1] and during angiogenesis-dependent diseases, including cancer [2]. In view of its essential contribution to physiological processes and major pathologies, angiogenesis is tightly controlled, mainly through the balance between the production and release of pro-angiogenic and antiangiogenic molecules (Figure 1).

Figure 1. The balance between the production and release of pro- and antiangiogenic molecules regulates neovascularization. For a more exhaustive list of antiangiogenic compounds and abbreviations, see Tables 1 and 2.

Pro-angiogenic molecules are a heterogeneous group of proteins that include the vascular endothelial growth factors (VEGFs) and fibroblast growth factors (FGFs). They induce angiogenesis by interacting with tyrosine kinase receptors (TKRs) expressed on the endothelial cell (EC) surface. A common theme among angiogenic growth factors (AGFs) is their interaction with (co)receptors other than TKRs (e.g. heparan sulfate proteoglycans (HSPGs) and integrins [3]). This complex pattern of extracellular interactions is mirrored by the intricacy of the signal transduction pathway(s) triggered by AGFs in ECs [4]. Once stimulated, ECs acquire the “angiogenic phenotype”, namely the ability to execute all the different steps of the angiogenic process, including extracellular matrix (ECM) degradation, a change of surface expression of adhesion molecules, proliferation, and chemotactic migration [4].
2. Antiangiogenic Compounds

Antiangiogenic compounds are a heterogeneous group of molecules that includes proteins, polysaccharides and glycosphingolipids found in the body fluids and ECM. A common theme among antiangiogenic compounds is their ability to directly bind and sequester AGFs in the extracellular environment, thus hampering their interaction with ECs (Table 1).

Table 1. Endogenous antiangiogenic molecules that bind and sequester AGFs in the extracellular environment. TSP-1 is highlighted in grey.

| Molecule                        | AGF bound                  | Reference                          |
|---------------------------------|----------------------------|------------------------------------|
| TSP-1                           | FGF2, VEGF, HGF, HIV-1 Tat, TGF-β₁ | [5–8], [9,10], [11], [12], [13] |
| α₂-macroglobulin                | FGF2, VEGF, TGF-β, IL8, TNF  | [14], [15], [16], [17], [18]      |
| heparin                         | FGF2, VEGF, HIV-1 Tat, HGF   | [19], [20], [21], [22]            |
| pentraxin-3 (PTX3)              | FGF2, FGF8                  | [23]                               |
| factor VII-activating protease  | FGF2, PDGF                  | [24], [25]                         |
| platelet factor 4 (PF-4)        | FGF2, VEGF                  | [26], [27]                         |
| SPARC                           | VEGF, PDGF                  | [28], [29]                         |
| CXCL13                          | FGF2                       | [30]                               |
| gangliosides                    | FGF2                       | [31]                               |
| fibronectin (fibronecin fragment)| FGF2                       | [32]                               |
| vitronectin                     | FGF2                       | [33]                               |
| soluble VEGF receptor (VEGFR)-1 | VEGF                       | [34]                               |
| ADAMTS1                         | VEGF                       | [35]                               |
| heparin affin regulatory peptide (HARP) | VEGF                 | [36]                               |
| connective tissue growth factor (CTGF) | VEGF            | [37]                               |
| soluble endoglin                | TGF-β₁                     | [38]                               |
| decorin                         | TGF-β₁                     | [39]                               |
| secretory component             | IL8                        | [40]                               |

However, under certain conditions, the direct interaction with a given ligand may lead to oligomerization of the AGF, its protection from proteolytic degradation and a rise in local concentration. This, in turn, will lead to proangiogenic rather than antiangiogenic effects, as already demonstrated for AGF binding to heparin, collagen and fibrinogen/fibrin (see [3] and references therein).

Besides binding AGFs, antiangiogenic molecules can inhibit angiogenesis by: i) inhibiting AGF production by tumor cells; ii) inhibiting surface expression of AGF-receptors on ECs; iii) binding (and masking) AGF receptors; iv) reducing EC responsiveness to AGFs (usually by engaging specific receptors that modify the EC phenotype); v) inhibiting effectors of angiogenesis produced by ECs (i.e., proteases required for ECM degradation) (Figure 2 and Table 2).

Two main considerations emerge from Table 1 and 2: i) some antiangiogenic molecules (including TSP-1) target different AGFs simultaneously; ii) some antiangiogenic molecules (including TSP-1) inhibit the angiogenic process through multiple mechanisms. These indications may provide useful suggestions for designing therapeutic strategies to inhibit angiogenesis, since pathological neovascularization is often the result of the simultaneous, non-redundant actions of various AGFs [41,42]. Inhibiting neovascularization by drugs directed against a single AGF/TKR presents several
limitations [41]. Moreover, developing drugs acting on multiple targets/mechanisms may limit the insurgence of drug resistance, which is a major problem with conventional antineoplastic therapies. TSP-1 interferes simultaneously with several AGFs (Table 1) through different mechanisms of action (Table 2), thus offering a paradigm for the development of antiangiogenic drugs. This review discusses TSP-1 and TSP-1-based inhibitors of angiogenesis, their mechanisms of action and therapeutic potential.

**Figure 2.** Action on angiogenesis. Antiangiogenic molecules affect AGFs by acting on AGF-producing cells, AGFs themselves, AGF receptors (AGFR), ECs, and angiogenesis effectors produced by activated ECs.

### 3. Structure and Biological Activity of TSP

The mammalian family of TSPs comprises five members, including TSP-1 and TSP-2, which form group A, homotrimeric TSPs. They are very similar in structure and can all inhibit angiogenesis, although they are expressed differently in various tissues during development and adulthood.

**Table 2.** Endogenous antiangiogenic molecules and their mechanisms of action. TSP-1 is evidenced in grey.

| MOLECULE | MECHANISM OF ACTION |
|----------|---------------------|
| **inhibition of AGF expression/release by producing cells** | |
| homocysteine | lowering FGF2 levels [43] |
| interleukin (IL)-12 | lowering FGF2 levels [44] |
| TSP-1 | lowering FGF2 levels [45] |
| **inhibition/interference with AGF receptors on ECs** | |
| IL-1, IFN-γ | TK-FGF receptors (TK-FGFR) down-regulation [46] |
| anosmin-1 | TK-FGFR occupancy [47] |
| thromboxane | inhibition of TK-FGFR1 internalization [48] |
| soluble form of TKR | formation of heterodimers with TK-FGFR1 [49] |
| antithrombin | HSPG down-regulation [50] |
| PF4 | HSPG occupancy [51], unknown [52] |
Table 2. Cont.

| MOLECULE                  | MECHANISM OF ACTION                                      |
|---------------------------|----------------------------------------------------------|
| endostatin                | HSPG occupancy [53]                                     |
| kallistatin               | HSPG occupancy [54]                                     |
| histidine-rich glycoprotein| HSPG occupancy [51]                                     |
| endosulfatases            | HSPG desulfation [55,56]                                |
| heparinase                | HSPG degradation [57]                                   |
| TSP-1                     | HSPG occupancy [9], integrin occupancy [48]             |
|                           | inhibition/interference with AGF-activated second messengers in ECs |
| heat-shock proteins 70 and 90 | down-regulation of pAkt, c-Raf-1 and ERK1/2 [58]    |
| sprouty proteins          | inhibition of TK-FGFR signalling [59]                  |
| homeobox gene GAX         | inhibition of NF-kB signalling [60]                    |
| semaphorin-3F             | inhibition of ERK1/2 signalling [61]                   |
| angiostatin [a plasminogen (Plg) fragment] | inhibition of ERK1/2 signalling [62] |
| ghrelin                   | inhibition of TKR/MAPK signalling [63]                 |
| lysophosphatidylcholine   | inhibition of ras/ERK1/2 signalling [64]                |
| pigment epithelium-derived factor | inhibition of Fyn signalling [65]               |
| TSP-1                     | inhibition of VEGF-mediated Akt signalling [66]        |
|                           | modification of EC apoptosis, phenotype, responsiveness to AGFs |
| cleaved HMW kininogen     | tropomyosin engagement [67]                            |
| IL-4                      | alteration of cell cycle [68]                           |
| kininostatin (kininogen fragment) | inhibition of cyclin D1 expression [69]             |
| vitamin D3-binding protein| CD36 engagement [70]                                   |
| endostatin                | Shb activation [71]                                     |
| histidine-rich glycoprotein| tropomyosin engagement [72]                            |
| endostatin                | cytoskeleton organization [73], Shb activation [71]    |
| TSP-1                     | TNF-α over-expression [74], CD36 engagement [66,75], apoptosis [45,66,74], ECM modification [76] |
|                           | inhibition/interference with angiogenesis effectors    |
| IL-12                     | inhibition of FGF-induced proteases [44]               |
| tissue inhibitor metalloproteinase (TIMP)-2, 4 | inhibition of FGF-induced proteases [77]             |
| kallistatin               | inhibition of FGF-induced proteases [54]               |
| TSP-1                     | inhibition of FGF-induced proteases [78], binding to matrix metalloproteinase-2 (MMP-2) [79] |
|                           | unknown mechanism of action                             |
| collagen I [80], alphastatin (fibrinogen fragment) [81], CXCL14 [82], IL-12 [83], IP-10 [84], vasostatin [85], vasculostatin (fragment of brain angiogenesis inhibitor-1) [86], TGF-β1 [87], TNFs [88], somatostatin [89], retinoids [90], apolipoprotein(a) [91], prolactin (16 kDa fragment) [92] | |

In view of the extensive literature, here we only report inhibitors of FGF2 as a prototypic AGF.

TSP-1 was the first endogenous inhibitor of angiogenesis to be identified [30,93]. Each TSP-1 monomer is formed by an N-terminal globular domain, followed by the coiled-coil oligomerization domain, a von Willebrand Factor type C procollagen domain, three properdin-like type I repeats, two epidermal growth factor-like type II repeats and a signature domain comprising a third type II repeat, the calcium-binding wire-type III repeats, and the lectin-like C-terminal globular domain [14] (Figure 3).

Type I repeats is a relatively small region commonly considered the main antiangiogenic site of TSP-1. Interestingly, this domain is also present in several other proteins, often giving them significant antiangiogenic activity [94,95].
Figure 3. Schematic representation of TSP-1 structure.

The region comprising the third type II repeats, the type III repeats and the C-terminal globular end is the most conserved region in the TSP family [96]. Its properties are affected by calcium. The cooperative binding of calcium ions is the main feature of type III repeats and profoundly affects the structure/availability of active sequences in the whole cassette. The type III repeats contain a cryptic integrin recognition motif RGD [97], two sequences that bind cathepsin G and neutrophil elastase [98], and binding sites for collagen V and FGF2. These binding sites become exposed only after drastic structural changes induced by a low calcium concentration or disulfide bond reduction, illustrating the importance of environmental conditions for the bioavailability and activity of these sequences [7,97–99]. TSP-1 is active both as a whole molecule and as fragments [100], a property shared by the matricellular PTX3 [23,101], whereas most endogenous angiogenesis inhibitors are fragments of larger molecules with no intrinsic antiangiogenic activity (such as fibronectin, kininogen and plasminogen (Tables 1 and 2).

Figure 4. Direct and indirect antiangiogenic actions of TSP-1. TSP-1 sequesters AGFs in the extracellular environment and masks various AGF receptors. TSP-1 also reduces EC responsiveness to AGFs and induces apoptosis by activating CD36. It binds matrix metalloproteinase-2 (MMP-2), favoring its clearance. Finally, it inhibits AGF production by tumor cells.
TSP-1 has an extremely complex, context-dependent effect on angiogenesis, reflecting the heterogeneity of its functional domains, each interacting with selected cell receptors, AGFs, ECM components, and proteases [100]. Thus, in a given biological setting, the local/temporal expression of these ligands and the availability of each TSP-1 domain drive the pattern of molecular interactions which, in turn, dictate the biological effects of TSP-1 [78]. As a consequence, TSP-1 exerts both pro- and antiangiogenic effects in vitro and in vivo depending on its concentration [102], free or ECM-associated status [103–105] and oligomerization [106–108].

TSP-1 affects angiogenesis both directly and indirectly. As a direct inhibitor, it interacts with specific receptors (i.e., CD36 and CD47 [109]) on ECs to affect apoptosis and functions related to angiogenesis. As an indirect inhibitor, it binds to and influences the activity/bioavailability of various mediators of angiogenesis, including AGFs, cytokines and proteases (Tables 1 and 2, Figure 4).

3.1. Direct effects of TSP-1

TSP directly affects ECs and tumor cells by interacting with specific receptors. CD36 was the first TSP-1 receptor identified [110]. It is an 88-kDa glycoprotein expressed by many cell types including ECs [111]. TSP-1 and CD36 interact through the CLESH-1 domain in CD36 and the type I repeats in TSP-1 [112]. CD36 constitutively associates with β1 integrins and VEGF receptor 2 (VEGFR-2) in ECs [113,114], with interesting implications for the cross-talk among TSP-1, CD36, VEGFR-2 and integrins in the antiangiogenic action of TSP-1. This interaction has manifold consequences: it inhibits FGF2-induced EC migration, morphological organization [112,113], production of nitric oxide (NO) [115] and angiogenesis in vivo [116], induces apoptosis in ECs [116] and tumor cells [117,118] and down-regulates the expression and phosphorylation of VEGFR-2 on EC surface [113,114].

CD47 was originally identified as the receptor that mediates cell adhesion and spreading to TSP-1 [119,120]. CD47 also forms a signalling complex with integrins [121]. By binding to CD47, TSP-1 inhibits NO-cGMP signalling, hence also neovascularization [115,122].

TSP-1 binds heparin and HSPGs (syndecan-1 and 4, perlecan, decorin) through its N-terminal domain (Table 3). This interaction can have antiangiogenic effects. TSP-1 displaces VEGF from EC HSPGs, inhibiting angiogenesis [9]. However, by binding to syndecan-4, TSP-1 can also exert angiogenic effects, protecting ECs from apoptosis and stimulating tubulogenesis [123]. The heparin binding motif Hep II also comprises the binding sequence for α6 integrin, pointing to cooperation between HSPGs and integrins, as already demonstrated for CD36 and CD47 (see above).

The low-density lipoprotein receptor-related protein (LRP) acts as a TSP-1 receptor. Like HSPGs, LRP can exert opposite effects on angiogenesis. It mediates endocytosis of the TSP-1/VEGF [124] and TSP-1/MMP-2 [79] complexes, contributing to the CD36-independent inhibition of VEGF angiogenic activity by TSP-1. However, the binding of TSP-1 to calreticulin (Table 3) enhances calreticulin binding to LRP that, in turn, induces EC motility and focal adhesion disassembly [125,126].

TSP interacts with several β1 integrins and with α5β3, involved in angiogenesis. Different binding sites for integrins have been mapped in TSP-1 (Table 3). Again, the various interactions between the different functional domains of TSP-1 and the different integrins can mediate both pro- [127] and antiangiogenic [128] effects. However, preclinical studies indicate that small TSP-1 peptides containing the integrin binding sites, as well as disintegrins or anti-integrin antibodies, block EC pro-
angiogenic functions such as adhesion, proliferation, survival, wound healing, motility and angiogenesis in vivo [127,129,130].

3.2. Indirect effects of TSP-1

Besides cell surface receptors, TSP-1 interacts with several other partners, including AGFs (Table 3). TSP-1 binds FGF2 with affinity similar to the FGF2/HSPG interaction. Accordingly, heparin prevents the TSP-1/FGF2 interaction and TSP-1 prevents the FGF2/HSPG interaction [5]. As a consequence of its interaction with FGF2, TSP-1 inhibits FGF2-triggered proliferation and chemotaxis in ECs [5–7]. Finally, TSP-1 prevents FGF2 accumulation in the ECM, favoring its mobilization as inactive TSP-1/FGF2 complexes [6]. These observations suggest that free TSP-1 acts as a scavenger for ECM-associated FGF2, affecting its location, bioavailability and function.

TSP-1 binds both free and cell-associated VEGF [9], suggesting that it regulates the bioavailability of VEGF in the microenvironment and its capacity to bind to its EC receptors during neovascularization. Also, TSP/VEGF complexes are internalized via LRP-1 [131]. This contributes to TSP-1’s ability to inhibit VEGF-induced EC tubulogenesis in vitro and angiogenesis in vivo [9].

Table 3. TSP-1 ligands and their binding domains in the TSP-1 structure.

| Ligand       | Binding domain in TSP-1                                      | Reference |
|--------------|-------------------------------------------------------------|-----------|
| AGFs         |                                                             |           |
| FGF2         | • type III repeats                                          | [7]       |
| VEGF         | • type I repeats                                           | [37]      |
| HGF          | • 3D conformation                                          | [11]      |
| HIV-1 Tat    | N.D.                                                        | [12]      |
| TGF-β        | • 2nd type I repeats (RFK sequence)                        | [132–134] |
|              | • type I repeats (WSXW sequence)                           | [133,134] |
| PDGF-BB      | • 3D conformation                                          | [135]     |
| MMP-2        | • type I repeats                                           | [136]     |
| Plg/plasmin  | N.D.                                                        | [137–139] |
| tissue Plg activator |                                                      | [140]      |
| urokinase Plg activator |                                      | [141]      |
| neutrophil elastase |                                                        | [142]      |
| cathepsin G  | • type III repeats                                          | [142,143] |
| tissue factor inhibitor |                                                   | [144]      |
| free molecules (body fluids) |                                                   |           |
| proteases and regulators |                                               |           |
| heparin      | • N-ter domain [motifs Hep I (aa 17-35) & Hep II (aa 78-94)] | [103,145] |
|              | • type I repeats                                           | [146,147] |
|              | • signature domain                                         | [148]     |
| histidine-rich glycoprotein |                                             | [149]      |
| factor V     | N.D.                                                        | [150]     |
| angiocidin   | • 2nd and 3rd type I repeats (CSVTCG sequence)             | [151]     |
| calumenin    | • N-ter domain (aa 21-228)                                 | [152]     |
| endostatin   | N.D.                                                        | [153]     |
TSP-1 binds HGF in a calcium-independent manner. Heat denaturation reduces its binding to HGF, suggesting that a proper 3D conformation is required [11]. Mature two-chain and precursor single-chain HGF both bind to TSP-1. Heparin prevents this interaction but does not disrupt established complexes. At a biological level, TSP-1 inhibits HGF-induced chemotaxis of ECs in vitro and HGF-induced angiogenesis in vivo [11].

TSP-1 binds HIV-1 Tat [12] inhibiting Tat-induced EC migration in vitro and angiogenesis in vivo [12,174]. It also binds and activates transforming growth factor (TGF)-β1 through sequences located in the type I repeats [132–134]. TSP-1-associated TGF-β1 is biologically active and protected from inactivation. As a result, the inhibition of ECs by TSP-1 is at least partly mediated by complexed TGF-β1 [13,134]. TSP-1 binds both free or substrate-associated PDGF-BB in a calcium-dependent way [135], but the biological importance of these interactions remains to be clarified.

As a matricellular protein, TSP-1 participates in organizing the ECM, which provides environmental and positional cues to ECs during angiogenesis. ECM components interact to form a

---

**Table 3. Cont.**

| Ligand               | Binding domain in TSP-1                                      | Reference           |
|----------------------|-------------------------------------------------------------|---------------------|
| CD36, CD47           | • type I repeats                                            | [112]               |
| HSPGs                | • N-ter domain [motifs Hep I (aa 17-35) & Hep II (aa 78-94)]| [103,145]           |
|                      | • signature domain                                          | [148]               |
| sulfated glycolipids | • N-ter domain                                              | [155]               |
|                      | • 3D conformation                                            | [155]               |
| LRP                  | • N-ter domain                                              | [126,155]           |
| VLDL receptor        | • N-ter domain                                              | [156,157]           |
| calreticulin         | • N-ter domain (aa 17-35)                                   | [126,158]           |
| integrins            | • N-ter domain                                              | [107,129,159–161]   |
|                      | • type I repeats                                            |                     |
|                      | • type III repeats (RGD sequence)                            | [128,130,162]       |
|                      | N.D., not determined.                                       |                     |
| collagen I           | N.D.                                                        | [164]               |
| collagen V           | • procollagen domain + type I & II repeats                  | [165,166]           |
| fibronectin          | • N-ter domain + type I & II repeats                        | [167,168]           |
| laminin              | N.D.                                                        | [165]               |
| fibrinogen/fibrin    | • N-ter domain                                              | [157]               |
|                      | • procollagen domain                                        | [146]               |
|                      | • type I repeats                                            | [169,170]           |
| von Willebrand factor| • signature domain                                          | [171]               |
| dermatan sulfate     | • N-ter domain (KKTR sequence)                              | [172]               |
| chondroitin sulfate  | • N-ter domain                                              | [155]               |
| IGF-binding protein-5| • N.D.                                                      | [173]               |

N.D., not determined. HSPGs, reported here as cell surface receptors, are also constituents of ECM. Conversely, dermatan- and chondroitin-sulfates, reported as ECM components, also exist as saccharidic chains of cell surface proteoglycans.
complex structural framework, and TSP-1 binds several of them (Table 3), with various consequences on ECM assembly and adherent EC behavior. Also, ECM is continuously remodeled by proteases. TSP-1 binds several proteases, including MMP-2, plasmin, neutrophil elastase and cathepsin G [79,98,136,175,176]. It promotes MMP-2 clearance via endocytosis by LRP [79,175] and suppresses MMP-3-mediated activation of proMMP-9 [177]. Conversely, it stimulates MMP-9 expression in ECs, promoting tubulogenesis [178]. The antiangiogenic 140 kDa TSP-1 fragment induces TIMP-2 over-expression [103], whereas the proangiogenic N-terminal domain of TSP-1 increases MMP-9 and MMP-2 release and reduces TIMP-2 expression by ECs.

Thus many of TSP-1’s effects on neovascularization are due to its ability to bind several molecules present in body fluids, ECMs, and the EC surface. It can therefore be envisaged at the centre of a complex interplay among AGFs, ECM components and their receptors and proteases. Through these multiple interactions, TSP-1 orchestrates their bioavailability, mutual binding and activities, leading to regulation of EC behavior during angiogenesis (Figure 5).

The “multi-binding” properties of TSP-1 depend on its modular structure in which several binding sequences are in close proximity, a feature that may lead to the formation of large multi-molecular complexes [140,149] in which the activity of each sequence becomes context-dependent, according to the environmental conditions and the predominant ligand. The ”multi-binding” capacity may also favor the coupling of some of its receptors (e.g. CD36, CD47, integrins, HSPGs and VEGFR-2, see above).

**Figure 5.** TSP-1 interactome. AGFs bind receptors inducing proteases that remodel ECM and mobilize AGFs, creating an environment favorable to EC proliferation and migration. TSP-1 binds several of these regulators, orchestrating their interactions/activities and leading to fine tuning of EC behavior during neovascularization.
4. Therapeutic Exploitation of TSP-1 as an Antiangiogenic Agent

With its multifaceted roles in the control of angiogenesis and oncogenesis, TSP-1 could be exploited therapeutically by different approaches.

4.1. TSP-1 upregulation

This is based on the observation that many antiangiogenic molecules act indirectly by upregulating the production of TSP-1. Thus, the simplest way to exploit TSP-1’s antiangiogenic potential would be to deliver molecules that induce its over-expression in producing cells (Table 4).

**Table 4.** Natural and synthetic molecules that induce over-expression of TSP-1.

| Molecule                                                                 | References |
|--------------------------------------------------------------------------|------------|
| glucose                                                                  | [179]      |
| peroxisome proliferator-activated receptor agonist fenofibrate           | [180]      |
| trichostatin-A                                                           | [181]      |
| retinoic acid                                                            | [182,183]  |
| somatostatin receptor subtype 2                                          | [10]       |
| cyclic adenosine 5’-monophosphate-activated guanine nucleotide exchange factor for Rap1 | [184] |
| angiostatin                                                              | [185]      |
| PHA -665752 (a small molecule, ATP-competitive inhibitor of c-Met receptor) | [186] |
| delta4-tibolone                                                          | [187]      |
| phorbol 12-myristate 13-acetate                                          | [183]      |
| fibulin-5                                                                | [188]      |
| angiotensin II and its agonist CGP42112A                                  | [189,190]  |
| endostatin                                                               | [191]      |
| estradiol                                                                | [192]      |
| progesterone and raloxifene                                               | [193]      |
| IL-6                                                                     | [183]      |
| IL-18                                                                    | [194]      |
| erythropoietin                                                           | [195]      |
| epidermal growth factor                                                  | [196]      |
| TFG-β1, FGF2                                                             | [197]      |
| thrombin                                                                 | [198]      |
| inhibitors of DNA methyltransferases and histone deacetylases            | [199]      |
| CD26-processed chemokines CXCL12 and CCL5                               | [200]      |

The practical exploitation of this approach might be hampered by the unpredictable effects of non-selective over-expression of such a pleiotropic molecule. Nonetheless, it is interesting to note that the antiangiogenic, antineoplastic activity of metronomic, low-dose cyclophosphamide has been associated with increased levels of TSP-1 [201,202]. Similarly, TSP-1 is induced in colon cancer models after treatment with 5-FU [203], in rat prostate tumors treated with cyclophosphamide, doxorubicin or paclitaxel [204], in head and neck squamous carcinoma cells treated with docetaxel [205], in neuroblastoma cells treated with valproic acid [206], and in HT-29 colon cancer xenografts treated with metronomic irinotecan [207]. Metronomic irinotecan also raises plasma levels and gene expression of TSP-1 in patients with metastatic colorectal cancer [208].
4.2. Gene therapy

More controlled TSP-1 over-expression could be achieved by gene therapy, whose advantages are schematized in Figure 6. TSP-1 over-expression can be obtained by targeting the TSP-1 gene itself or a number of oncogenes/oncosuppressor genes that influence its expression. Adams and co-workers [106] nicely demonstrated the versatility of the gene therapy approach by using different TSP-1 modules differing in their capacity to selectively interact with various ligands.

**Figure 6.** Advantages of TSP-1-based gene therapy. By different strategies (1) and by targeting different cell types (4), it is possible to induce directly the expression of the TSP-1 gene, to stimulate or inhibit the expression of TSP-1 enhancers/inhibitors (2) or to express selected TSP-1 modules (3).

The list below describes some TSP-1-based gene therapy strategies:

i) Fibroblasts retrovirally transduced to produce high levels of TSP-1 resulted in high levels of the protein that inhibited angiogenesis and tumor growth in different models [209];

ii) recombinant adeno-associated virus (AAV)-mediated delivery of the three type I repeats (3TSR) resulted in expression of the transgene in normal tissues, reduced VEGF-induced angiogenesis, reduced tumor growth and microvessel density both locally and at distant sites [210];

iii) AAV-mediated gene therapy has also been exploited to express a TSP-1 fragment that inhibits human leukemia xenografts growth in nude mice [211].

iv) expression of 3TSR or of the second type I repeats containing the TGF-β-activating sequences significantly inhibited in vivo tumor angiogenesis and growth in nude mice [212];

v) expression of TSP-1-derived 4N1K peptide-containing proteins in renal cell carcinoma tissues was associated with a decrease in tumor growth and angiogenesis [213];

vi) transfection of a TSP-1 complementary cDNA antisense into glioblastoma cells lines significantly reduced TSP-1 production and cell motility [214];

vii) p53 inactivation lowered TSP-1 production [215,216]. Accordingly, topical delivery of p53 DNA to the lung increased TSP-1 expression, reduced microvessel density and limited lung tumor burden, prolonging the survival of tumor-bearing mice [217];

viii) c-Myc-regulated cluster miRNA-17-92, over-expressed in many human cancers, inhibited TSP-1 expression in cancer.
cells and in ECs. Inhibition of miR-17-92 by means of microRNAs increased TSP-1 expression and reduced VEGF-induced EC proliferation, migration and morphogenesis [218,219]; ix) transfection of c-Jun and/or RARalpha expression vectors into hepatoma cells and ECs raised mRNA and protein levels of TSP-1 [183]; x) over-expression of connexin-26 in human breast tumor cells up-regulated both the transcription and translation of TSP-1, retarding tumor growth \textit{in vivo} [220]; xi) knockdown of Her-2/neu expression by siRNA increased the expression of TSP-1 and reduced that of VEGF [221]; xii) silencing CD26 using siRNA increased TSP-1 expression in T cells [200]. As discussed below, over-expression of TSP-1 or related molecules can sometimes lead to an increase in tumor growth, as after over-expression of the TSP-1 fragment 167-569 in C6 glioma cells [222].

4.3. TSP-1-based peptides and peptidomimetics

The use of TSP-1-based drugs must deal with the fact that TSP-1, like its related peptides, can elicit both anti- and pro-angiogenic responses. However, this expands, rather than limits, the possibility of developing TSP-1-based antiangiogenic therapies by designing drugs or gene therapies that mimic the antiangiogenic effects of TSP-1 or antagonize the pro-angiogenic ones (Figure 7).

The use of biologically relevant, functional protein sequences as an entry point for the development of novel lead compounds is a powerful tool in drug discovery [223–226]. The starting sequences can elucidate the roles of key interactions (hot spots) in the regulation of important protein-protein interactions that a drug must agonize or antagonize. This knowledge may then boost our ability to interfere with specific pathological interactions, providing attractive therapeutic opportunities and extending medicinal chemistry to new classes of compounds.

\textbf{Figure 7.} Design of anti-angiogenic TSP-1 peptides/peptidomimetics
4.3.1. Characterization of TSP-1 active domains and sequences

The design of TSP-1-based drugs started from the identification/characterization of TSP-1 active domains. These studies used antibodies directed against the various portions of TSP-1 or peptides representing various TSP-1 fragments. The latter can be obtained by controlled proteolytic digestion of the intact TSP-1 protein [227] (e.g. by ADAMTS-1 [228] or thrombin [103]) or, more often, by the production of recombinant fragments [100]. Table 5 lists the TSP-1-derived peptides studied so far for their ability to regulate neovascularization. The pro-angiogenic domains of TSP-1 are mostly mapped in its N-terminal domain, while the main antiangiogenic sequences are in the second and third type I repeats.

Besides ECs, TSP-1-derived peptides also act on tumor cells. A peptide representing the Hep-I sequence induced promyelocytic leukemia cell differentiation and apoptosis [229], while 3TSR inhibited proliferation and induced apoptosis of B16F10 tumor cells in a TGF-β-dependent manner [114] in vitro and reduced tumor growth in orthotopic pancreatic xenografts [230,231] and in a model of polyoma middle T transgenic mice [232]. The peptide GGWSHW, located within the type I repeats, induces promyelocytic leukemia cell differentiation and apoptosis [229]. Retro-inverso peptides of the WSHWSxPWS sequence (aa sequence 438–452) inhibit the growth of MDA-MB-435 carcinoma cells in the mammary fat pad of nude mice [100,233]. WSxW-containing peptides also inhibit TSP-1-mediated activation of the TGF-β latent complex [133] and motility of glioma cells [214].

### Table 5. Pro- and antiangiogenic TSP-1-derived peptides.

| Pro-angiogenic peptides              | Mechanism                                                                 | Reference   |
|--------------------------------------|---------------------------------------------------------------------------|-------------|
| peptides from the N-ter domain       | • increase proteolytic activity of EC                                      | [78,103,129]|
|                                      | • interaction with integrins, HSPG, LRP                                   |             |
|                                      | • disassembly of focal adhesion and EC migration                          |             |
| **antiangiogenic peptides**          |                                                                           |             |
| integrin-binding sequence of the N-ter domain | • $\alpha_3$$\beta_1$, integrin antagonists                            | [129]       |
| sequences in the pro-collagen domain | • various                                                               | [227]       |
| various peptides from the second and third type I repeats | • CD36-mediated EC apoptosis                                             | [228,234,235]|
|                                      | • inhibition of EC response to AGF                                      |             |
|                                      | • binding to protein/glycosaminoglycans                                  |             |
|                                      | • TGF-β-activation                                                      |             |
|                                      | • integrin antagonist                                                    |             |
| peptide from the type III repeats    | • FGF-2 binding and sequestration                                       | [8]         |
| peptide 4N1 in the C-ter domain      | • CD47 binding                                                          | [122]       |

A fragment spanning the type III repeats and C-terminal domain causes promyelocytic leukemia NB4 cell death through CD47/$\alpha_v$$\beta_3$ [118]. Peptides containing the second type I repeats also inhibit tumor growth by regulating tumor cell proliferation and apoptosis in a TGF-β-dependent manner. Their capacity to inhibit angiogenesis is instead TGFΦ–β–independent [134]. Finally, peptide 4N1, interacting with CD47, induces apoptosis in different breast cancer cell lines [236] and sensitizes human prostate tumor cells to taxane cytotoxicity [237], though it protects normal cells from apoptosis [238].
4.3.2. Modifications of TSP-1-derived peptides and generation of peptidomimetics

The therapeutic exploitation of synthetic peptides is limited by their well-known shortcomings: unfavorable pharmacodynamics/pharmacokinetics (poor oral bioavailability and/or short duration of action), lack of receptor selectivity and low affinity (with $K_d$ in the mM-$\mu$M range, compared to the pM-nM range of the $K_d$ for parent proteins [239]). Modifying the peptide structure by acylation, PEGylation, fatty acid acylation, unnatural amino acids or restricted conformation can largely overcome these limits [240]. Many TSP-1 sequences exploitable for the design of antiangiogenic drugs are exposed in the TSP-1 molecule only after drastic structural changes induced by a low calcium concentration, different ligands, or reduction of disulfide bonds, indicating the structural modifications that must be introduced (and maintained) in the derived peptides. Guo and coworkers synthesized stereospecific analogs of the KRFKQDGGWSH/WSPWSSC peptide from TSP-1 type I repeats that allowed dissection of different biological properties of the peptide enhancing the desirable ones [233]. Similarly, the peptide D-reverse amKRFKQDGGWSH-WSPWSSac inhibits proliferation of C6 glioma cells in vitro and tumor growth in vivo [241]. Other modified TSP-1 peptides have been described: CVX-22 is a chimera obtained by fusing two mimetic nonamer peptides from TSP-1 type I repeats to the Fab binding site of a humanized scaffold antibody. This chimera selectively induces apoptosis of VEGFR-2-positive ECs in melanoma [242]. A phase I trial for this compound found some possibly drug-related adverse events but no dose-limiting toxicities [243].

ABT-510 is an antiangiogenic TSP-1 modified nonapeptide designed on the 7-mer active sequence GVITRIR of the second type I repeats. Although not active in the native conformation, appropriate modifications gave it strong antiangiogenic activity [75]. In detail, the first L-ile residue of the GVITRIR sequence was substituted with D-ile and the first Arg with the non-natural amino acid norvaline. The resulting D-enantiomer was capped by the addition of sarcosine at the N-terminus and proline ethylamide at the C-terminus, generating peptide ABT-526 [75]. A more soluble version of the peptide, eventually named ABT-510, was obtained by substituting D-ile with D-allo-ile [244,245]. Since its original preparation and description in 1999 [75], the antiangiogenic and antineoplastic activity of ABT-510 has been thoroughly investigated in vitro, in vivo and in humans, demonstrating that it has a favorable potency, solubility and pharmacodynamics/pharmacokinetics profile [245]. In ECs, it inhibits proliferation and migration, induces CD36-dependent apoptosis, and up-regulates CD95L/FasL. Besides acting on ECs, ABT-510 also induces apoptosis of CD36-expressing tumor cells [246], suggesting a double effect on both the vascular and tumor compartments, an important property of TSP-1 and related reagents that has been already mentioned above and that will be discussed further in this review.

In vivo ABT-510 inhibits angiogenesis in different assays [244,245,247,248]. It reduces tumor growth and microvessel density in a ras-dependent/VEGF-independent tumor model [249], in syngeneic and xenograft gliomas [247], in orthotopic bladder cancer [244] and ovarian carcinoma xenografts [246] and in Lewis lung carcinoma [245]. Besides tumor growth, ABT-510 also inhibits metastasis in the B16F10 model [244] and ovarian cancer xenografts [246]. It has shown promising single-agent activity in canine cancer, inducing objective responses and disease stabilization [250].

On the basis of these favorable preclinical data, ABT-510 was tested, as a single agent, in three phase I and 4 phase II clinical trials between 2005 and 2008. Although phase I studies indicated that
ABT-510 was safe and had a good toxicity profile even after several months’ use with different schedules [251–253], it showed little clinical activity on renal cell carcinoma, soft tissue sarcoma and melanoma [252–255]. These disappointing results, however, were no different from those already seen with other antiangiogenic agents used as single agents [253], justifying further evaluation in combination therapies [254,255]. Preclinical studies with the combination of ABT-510 and valproic acid [256] or CeeNu [250] gave favorable results and two phase I trials demonstrated the safety of ABT-510 in combination with chemotherapeutics such as gemcitabine, cisplatin and 5-FU/leucovorin [257,258].

A completely different and innovative rational approach that may overcome the limits of peptide-based antiangiogenic therapy is the identification of synthetic, non-peptidic molecules that mimic the hot-spot interactions in macromolecular complexes [224,259,260]. By using a peptide array approach followed by binding assays with synthetic peptides and recombinant proteins, we identified a FGF2 binding sequence of TSP-1 in the 15mer sequence DDDDDNDKIPDDRDN of the type III repeats. The peptide itself did not inhibit FGF2 function but served as a tool to identify the physico-chemical determinants of FGF2 recognition by TSP-1 and to design non-peptidic inhibitors. Nuclear magnetic resonance and molecular dynamics simulations taking into account the full flexibility of the ligand and receptor identified the relevant residues and conformational determinants for the peptide-FGF interaction. This information was translated into a pharmacophore model used to screen the NCI2003 small molecule databases, leading to the identification of three small molecules that bound FGF2 with affinities in the nanomolar range of concentration. These compounds prevented FGF2 binding to ECs, and inhibited FGF2-induced EC proliferation in vitro, and angiogenesis in the CAM assay. Although the lead compounds have still to be derivatized to improve the drug-like properties before they can be considered real drug candidates, our study show that it is feasible to develop small molecule mimics of TSP-1, and more in general of endogenous proteins, as therapeutic agents [8].

5. Conclusions

Among all the endogenous inhibitors of angiogenesis, TSP-1 seems the most promising for the development of efficacious antiangiogenic/antineoplastic therapies. TSP-1 can act with different mechanisms on different targets at cellular (leukocytes, endothelial, tumor and stromal cells) and molecular (AGFs, cell surface receptors, ECM) levels. Thus, TSP-1 can inhibit tumor progression not only through its well-known antiangiogenic action. It can induce an antineoplastic immune response by recruiting macrophages in the tumor and enhancing their cytotoxicity towards the tumor [261]. TSP-1 can also inhibit megakaryocytopoiesis [262] and coagulation [144,150,171], suggesting that its appropriate exploitation might help preventing the thromboembolic disorders that contribute to the morbidity/mortality of oncological patients [263]. Finally, and perhaps most importantly, TSP-1 can act directly on cancer cells, reducing their growth, inducing apoptosis [10,117,134,264–266], increasing their sensitivity to chemotherapeutics [237], and preventing metastatic dissemination [244,246]. Thus, TSP-1 can be positioned at the crossroads between tumor growth, angiogenesis, immunity and coagulation (Figure 8), extending its possibilities for therapeutic exploitation.

However, TSP-1 can exert opposite effects on the immune response against tumors, as demonstrated by the fact that it inhibited TCR-mediated T lymphocyte early activation [267]. Also,
peptide 4N1K induces cell death of monocytes and monocyte-derived DCs [268]. Paradigmatic of these divergent effects on the immune response is the observation that the absence of TSP-1 in “knock-out” mice can either increase [269] or attenuate [270] Th17 response. Also, while the TSP-1-N-terminal domain renders DCs phagocytic, the TSP-1-C-terminal domain causes a tolerizing phenotype in the same cells [271]. Thus, while the intact TSP-1 molecule inhibits phorbol myristate acetate/LPS-induced homotypic aggregation of human monocytes, the 70-kDa fragment of TSP-1 generated by proteolytic cleavage promotes homotypic aggregation [272]. Similarly, TSP-1 or its peptides can enhance thrombosis, instead of inhibiting it [150,273,274]. Finally, TSP-1 and the peptides can actually increase tumorigenicity [102,275–278] [192,222,279,280].

**Figure 8.** TSP-1 interferes with tumor progression at different levels. It blocks neovascularization, thus inhibiting tumor growth and metastasis which are further inhibited by its direct action on tumor cells. By acting on immune cells, TSP-1 may enhance the immune antineoplastic response. Finally, through its action on coagulation, TSP-1 may control the thromboembolic events that afflict oncological patients.

In conclusion, TSP-1 will remain an interesting source of therapeutic molecules for a variety of different applications, once the limits imposed by its structural and functional complexity are overcome by identification of the specific active sequence(s) and their proper exploitation.

**Acknowledgements**

Part of the work presented here was supported by grants from: Fondazione CARIPLO (MR, GT, MP), Istituto Superiore di Sanità, Progetto Nazionale AIDS (MR), Ministero dell’Istruzione, Università e Ricerca (MR, MP), Ministero della Salute (GT), Associazione Italiana per la Ricerca sul Cancro (MP, GT), Fondazione Berlucchi (MP).
References

1. Carmeliet, P.; Jain, R.K. Angiogenesis in cancer and other diseases. *Nature* **2000**, *407*, 249–257.
2. Folkman, J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.* **1995**, *1*, 27–31.
3. Rusnati, M.; Presta, M. Extracellular angiogenic growth factor interactions: an angiogenesis interactome survey. *Endothelium* **2006**, *13*, 93–111.
4. Presta, M.; Dell’Era, P.; Mitola, S.; Moroni, E.; Ronca, R.; Rusnati, M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev.* **2005**, *16*, 159–178.
5. Taraboletti, G.; Belotti, D.; Borsotti, P.; Vergani, V.; Rusnati, M.; Presta, M.; Giavazzi, R. The 140-kilodalton antiangiogenic fragment of thrombospondin-1 binds to basic fibroblast growth factor. *Cell Growth Differ.* **1997**, *8*, 471–479.
6. Margosio, B.; Marchetti, D.; Vergani, V.; Giavazzi, R.; Rusnati, M.; Presta, M.; Taraboletti, G. Thrombospondin 1 as a scavenger for matrix-associated fibroblast growth factor 2. *Blood* **2003**, *102*, 4399–4406.
7. Margosio, B.; Rusnati, M.; Bonezzi, K.; Cordes, B.L.; Annis, D.S.; Urbinati, C.; Giavazzi, R.; Presta, M.; Ribatti, D.; Mosher, D.F.; Taraboletti, G. Fibroblast growth factor-2 binding to the thrombospondin-1 type III repeats, a novel antiangiogenic domain. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 700–709.
8. Colombo, G.; Margosio, B.; Ragona, L.; Neves, M.; Bonifacio, S.; Annis, D.S.; Stravalaci, M.; Tomaselli, S.; Giavazzi, R.; Rusnati, M.; Presta, M.; Zetta, L.; Mosher, D.F.; Ribatti, D.; Gobbi, M.; Taraboletti, G. Non-peptidic thrombospondin-1-mimics as fibroblast growth factor-2 inhibitors: an integrated strategy for the development of new antiangiogenic compounds. *J. Biol. Chem.* **2010**, in press.
9. Gupta, K.; Gupta, P.; Wild, R.; Ramakrishnan, S.; Hebbel, R.P. Binding and displacement of vascular endothelial growth factor (VEGF) by thrombospondin: effect on human microvascular endothelial cell proliferation and angiogenesis. *Angiogenesis* **1999**, *3*, 147–158.
10. Laklai, H.; Laval, S.; Dumartin, L.; Rochaix, P.; Hagedorn, M.; Bikfalvi, A.; Le Guellec, S.; Delisle, M.B.; Schally, A.V.; Susini, C.; Pyronnet, S.; Bousquet, C. Thrombospondin-1 is a critical effector of oncosuppressive activity of sst2 somatostatin receptor on pancreatic cancer. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 17769–17774.
11. Lamszus, K.; Joseph, A.; Jin, L.; Yao, Y.; Chowdhury, S.; Fuchs, A.; Polverini, P.J.; Goldberg, I.D.; Rosen, E.M. Scatter factor binds to thrombospondin and other extracellular matrix components. *Am. J. Pathol.* **1996**, *149*, 805–819.
12. Rusnati, M.; Taraboletti, G.; Urbinati, C.; Tulipano, G.; Giuliani, R.; Molinari-Tosatti, M.P.; Sennino, B.; Giacca, M.; Tyagi, M.; Albini, A.; Noonan, D.; Giavazzi, R.; Presta, M. Thrombospondin-1/HIV-1 tat protein interaction: modulation of the biological activity of extracellular Tat. *FASEB J.* **2000**, *14*, 1917–1930.
13. Murphy-Ullrich, J.E.; Schultz-Cherry, S.; Hook, M. Transforming growth factor-beta complexes with thrombospondin. *Mol. Biol. Cell* **1992**, *3*, 181–188.
14. Asplin, I.R.; Wu, S.M.; Mathew, S.; Bhattacharjee, G.; Pizzo, S.V. Differential regulation of the fibroblast growth factor (FGF) family by alpha(2)-macroglobulin: evidence for selective modulation of FGF-2-induced angiogenesis. *Blood* **2001**, *97*, 3450–3457.

15. Bhattacharjee, G.; Asplin, I.R.; Wu, S.M.; Gawdi, G.; Pizzo, S.V. The conformation-dependent interaction of alpha 2-macroglobulin with vascular endothelial growth factor. A novel mechanism of alpha 2-macroglobulin/growth factor binding. *J. Biol. Chem.* **2000**, *275*, 26806–26811.

16. Feige, J.J.; Negoescu, A.; Keramidas, M.; Souchelnitskiy, S.; Chambaz, E.M. Alpha 2-macroglobulin: a binding protein for transforming growth factor-beta and various cytokines. *Horm. Res.* **1996**, *45*, 227–232.

17. Kurdowska, A.; Alden, S.M.; Noble, J.M.; Stevens, M.D.; Carr, F.K. Involvement of alpha-2-macroglobulin receptor in clearance of interleukin 8-alpha-2-macroglobulin complexes by human alveolar macrophages. *Cytokine* **2000**, *12*, 1046–1053.

18. LaMarre, J.; Wollenberg, G.K.; Gionias, S.L.; Hayes, M.A. Cytokine binding and clearance properties of proteinase-activated alpha 2-macroglobulins. *Lab. Invest.* **1991**, *65*, 3–14.

19. Rusnati, M.; Presta, M. Interaction of angiogenic basic fibroblast growth factor with endothelial cell heparan sulfate proteoglycans. Biological implications in neovascularization. *Int. J. Clin. Lab. Res.* **1996**, *26*, 15–23.

20. Norrby, K. 2.5 kDa and 5.0 kDa heparin fragments specifically inhibit microvessel sprouting and network formation in VEGF165-mediated mammalian angiogenesis. *Int. J. Exp. Pathol.* **2000**, *81*, 191–198.

21. Rusnati, M.; Coltrini, D.; Oreste, P.; Zoppetti, G.; Albini, A.; Noonan, D.; d'Adda di Fagagna, F.; Giacca, M.; Presta, M. Interaction of HIV-1 Tat protein with heparin. Role of the backbone structure, sulfation, and size. *J. Biol. Chem.* **1997**, *272*, 11313–11320.

22. Lietha, D.; Chirgadze, D.Y.; Mulloy, B.; Blundell, T.L.; Gherardi, E. Crystal structures of NK1-heparin complexes reveal the basis for NK1 activity and enable engineering of potent agonists of the MET receptor. *Embo. J.* **2001**, *20*, 5543–5555.

23. Rusnati, M.; Camozzi, M.; Moroni, E.; Bottazzi, B.; Peri, G.; Indraccolo, S.; Amadori, A.; Mantovani, A.; Presta, M. Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. *Blood* **2004**, *104*, 92–99.

24. Shibamiya, A.; Muhl, L.; Tannert-Otto, S.; Preissner, K.T.; Kanse, S.M. Nucleic acids potentiate Factor VII-activating protease (FSAP)-mediated cleavage of platelet-derived growth factor-BB and inhibition of vascular smooth muscle cell proliferation. *Biochem. J.* **2007**, *404*, 45–50.

25. Etscheid, M.; Beer, N.; Kress, J.A.; Seitz, R.; Dodt, J. Inhibition of bFGF/EGF-dependent endothelial cell proliferation by the hyaluronan-binding protease from human plasma. *Eur. J. Cell Biol.* **2004**, *82*, 597–604.

26. Lozano, R.M.; Redondo-Horcajo, M.; Jimenez, M.A.; Zilberberg, L.; Cuevas, P.; Bikfalvi, A.; Rico, M.; Gimenez-Gallego, G. Solution structure and interaction with basic and acidic fibroblast growth factor of a 3-kDa human platelet factor-4 fragment with antiangiogenic activity. *J. Biol. Chem.* **2001**, *276*, 35723–35734.

27. Bikfalvi, A. Platelet factor 4: an inhibitor of angiogenesis. *Semin. Thromb. Hemost.* **2004**, *30*, 379–385.
28. Kupprion, C.; Motamed, K.; Sage, E.H. SPARC (BM-40, osteonectin) inhibits the mitogenic effect of vascular endothelial growth factor on microvascular endothelial cells. *J. Biol. Chem.* **1998**, *273*, 29635–29640.

29. Raines, E.W.; Lane, T.F.; Iruela-Arispe, M.L.; Ross, R.; Sage, E.H. The extracellular glycoprotein SPARC interacts with platelet-derived growth factor (PDGF)-AB and -BB and inhibits the binding of PDGF to its receptors. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 1281–1285.

30. Spinetti, G.; Camarda, G.; Bernardini, G.; Romano Di Peppe, S.; Capogrossi, M.C.; Napolitano, M. The chemokine CXCL13 (BCA-1) inhibits FGF-2 effects on endothelial cells. *Biochem. Biophys. Res. Commun.* **2001**, *289*, 19–24.

31. Rusnati, M.; Tanghetti, E.; Urbanini, C.; Tulipano, G.; Marchesini, S.; Ziche, M.; Presta, M. Interaction of fibroblast growth factor-2 (FGF-2) with free gangliosides: biochemical characterization and biological consequences in endothelial cell cultures. *Mol. Biol. Cell* **1999**, *10*, 313–327.

32. Bossard, C.; Van den Berghe, L.; Laurell, H.; Castano, C.; Cerutti, M.; Prats, A.C.; Prats, H. Antiangiogenic properties of fibstatin, an extracellular FGF-2-binding polypeptide. *Cancer Res.* **2004**, *64*, 7507–7512.

33. Hollier, B.; Harkin, D.G.; Leavesley, D.; Upton, Z. Responses of keratinocytes to substrate-bound vitronectin: growth factor complexes. *Exp. Cell Res.* **2005**, *305*, 221–232.

34. Shibuya, M. Vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1): a dual regulator for angiogenesis. *Angiogenesis* **2006**, *9*, 225–230; discussion 231.

35. Luque, A.; Carpizo, D.R.; Iruela-Arispe, M.L. ADAMTS1/METH1 inhibits endothelial cell proliferation by direct binding and sequestration of VEGF165. *J. Biol. Chem.* **2003**, *278*, 23656–23665.

36. Heroult, M.; Bernard-Pierrot, I.; Delbe, J.; Hamma-Kourbali, Y.; Katsoris, P.; Barritault, D.; Papadimitriou, E.; Plouet, J.; Courty, J. Heparin affin regulatory peptide binds to vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. *Oncogene* **2004**, *23*, 1745–1753.

37. Venkatesha, S.; Toporsian, M.; Lam, C.; Hanai, J.; Mammoth, T.; Kim, Y.M.; Bdolah, Y.; Lim, K.H.; Yuan, H.T.; Libermann, T.A.; Stillman, I.E.; Roberts, D.; D'Amore, P.A.; Epstein, F.H.; Sellke, F.W.; Romero, R.; Sukhatme, V.P.; Letarte, M.; Karumanchi, S.A. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat. Med.* **2006**, *12*, 642–649.

38. O'Connor-McCourt, M.D.; Wakefield, L.M. Latent transforming growth factor-beta in serum: A specific complex with alpha 2-macroglobulin. *J. Biol. Chem.* **1987**, *262*, 14090–14099.
42. Barthlen, W.; Flaadt, D.; Girgert, R.; Conzelmann, J.; Schweizer, P.; Zugmaier, G.; Buck, M.; Knabbe, C. Significance of heparin-binding growth factor expression on cells of solid pediatric tumors. *J. Pediatr. Surg.* 2003, 38, 1296–1304.

43. Chang, P.Y.; Lu, S.C.; Lee, C.M.; Chen, Y.J.; Dugan, T.A.; Huang, W.H.; Chang, S.F.; Liao, W.S.; Chen, C.H.; Lee, Y.T. Homocysteine inhibits arterial endothelial cell growth through transcriptional downregulation of fibroblast growth factor-2 involving G protein and DNA methylation. *Circ. Res.* 2008, 102, 933–941.

44. Meeran, S.M.; Katiyar, S.; Elmets, C.A.; Katiyar, S.K. Interleukin-12 deficiency is permissive for angiogenesis in UV radiation-induced skin tumors. *Cancer Res.* 2007, 67, 3785–3793.

45. Zak, S.; Treven, J.; Nash, N.; Gutierrez, L.S. Lack of thrombospondin-1 increases angiogenesis in a model of chronic inflammatory bowel disease. *Int. J. Colorectal Dis.* 2008, 23, 297–304.

46. Norioka, K.; Mitaka, T.; Mochizuki, Y.; Hara, M.; Kawagoe, M.; Nakamura, H. Interaction of interleukin-1 and interferon-gamma on fibroblast growth factor-induced angiogenesis. *Jpn. J. Cancer Res.* 1994, 85, 522–529.

47. Hu, Y.; Guimond, S.E.; Travers, P.; Cadman, S.; Hohenester, E.; Turnbull, J.E.; Kim, S.H.; Bouloux, P.M. Novel mechanisms of fibroblast growth factor receptor 1 regulation by extracellular matrix protein anosmin-1. *J. Biol. Chem.* 2009, 284, 29905–29920.

48. Ashton, A.W.; Cheng, Y.; Helisch, A.; Ware, J.A. Thromboxane A2 receptor agonists antagonize the proangiogenic effects of fibroblast growth factor-2: role of receptor internalization, thrombospondin-1, and alpha(v)beta3. *Circ. Res.* 2004, 94, 735–742.

49. Ueno, H.; Gunn, M.; Dell, K.; Tseng, A., Jr.; Williams, L. A truncated form of fibroblast growth factor receptor 1 inhibits signal transduction by multiple types of fibroblast growth factor receptor. *J. Biol. Chem.* 1992, 267, 1470–1476.

50. Zhang, W.; Chuang, Y.J.; Swanson, R.; Li, J.; Seo, K.; Leung, L.; Lau, L.F.; Olson, S.T. Antiangiogenic antithrombin down-regulates the expression of the proangiogenic heparan sulfate proteoglycan, perlecan, in endothelial cells. *Blood* 2004, 103, 1185–1191.

51. Brown, K.J.; Parish, C.R. Histidine-rich glycoprotein and platelet factor 4 mask heparan sulfate proteoglycans recognized by acidic and basic fibroblast growth factor. *Biochemistry* 1994, 33, 13918–13927.

52. Sulpice, E.; Bryckaert, M.; Lacour, J.; Contreres, J.O.; Tobelem, G. Platelet factor 4 inhibits FGF2-induced endothelial cell proliferation via the extracellular signal-regulated kinase pathway but not by the phosphatidylinositol 3-kinase pathway. *Blood* 2002, 100, 3087–3094.

53. Nyberg, P.; Xie, L.; Kalluri, R. Endogenous inhibitors of angiogenesis. *Cancer Res.* 2005, 65, 3967–3979.

54. Miao, R.Q.; Agata, J.; Chao, L.; Chao, J. Kallistatin is a new inhibitor of angiogenesis and tumor growth. *Blood* 2002, 100, 3245–3252.

55. Wang, S.; Ai, X.; Freeman, S.D.; Pownall, M.E.; Lu, Q.; Kessler, D.S.; Emerson, C.P., Jr. QSulf1, a heparan sulfate 6-O-endosulfatase, inhibits fibroblast growth factor signaling in mesoderm induction and angiogenesis. *Proc. Natl. Acad. Sci. USA* 2004, 101, 4833–4838.

56. Lai, J.P.; Sandhu, D.S.; Shire, A.M.; Roberts, L.R. The tumor suppressor function of human sulfatase 1 (SULF1) in carcinogenesis. *J. Gastrointest. Cancer* 2008, 39, 149–158.
57. Chua, C.C.; Rahimi, N.; Forsten-Williams, K.; Nugent, M.A. Heparan sulfate proteoglycans function as receptors for fibroblast growth factor-2 activation of extracellular signal-regulated kinases 1 and 2. *Circ. Res.* **2004**, *94*, 316–323.

58. Kaur, G.; Belotti, D.; Burger, A.M.; Fisher-Nielson, K.; Borsotti, P.; Riccardi, E.; Thillainathan, J.; Hollingshead, M.; Sausville, E.A.; Giavazzi, R. Antiangiogenic properties of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin: an orally bioavailable heat shock protein 90 modulator. *Clin. Cancer Res.* **2004**, *10*, 4813–4821.

59. Hanafusa, H.; Torii, S.; Yasunaga, T.; Nishida, E. Sprouty1 and Sprouty2 provide a control mechanism for the Ras/MAPK signalling pathway. *Nat. Cell Biol.* **2002**, *4*, 850–858.

60. Patel, S.; Leal, A.D.; Gorski, D.H. The homeobox gene Gax inhibits angiogenesis through inhibition of nuclear factor-kappaB-dependent endothelial cell gene expression. *Cancer Res.* **2005**, *65*, 1414–1424.

61. Kessler, O.; Shraga-Heled, N.; Lange, T.; Gutmann-Raviv, N.; Sabo, E.; Baruch, L.; Machluf, M.; Neufeld, G. Semaphorin-3F is an inhibitor of tumor angiogenesis. *Cancer Res.* **2004**, *64*, 1008–1015.

62. Redlitz, A.; Daum, G.; Sage, E.H. Angiostatin diminishes activation of the mitogen-activated protein kinases ERK-1 and ERK-2 in human dermal microvascular endothelial cells. *J. Vasc. Res.* **1999**, *36*, 28–34.

63. Baiguera, S.; Conconi, M.T.; Guidolin, D.; Mazzocchi, G.; Malendowicz, L.K.; Parnigotto, P.P.; Spinazzi, R.; Nussdorfer, G.G. Ghrelin inhibits angiogenic activity of rat brain microvascular endothelial cells. *J. Vasc. Res.* **2004**, *14*, 849–854.

64. Rokitake, Y.; Kawashima, S.; Yamashita, T.; Ueyama, T.; Ishido, S.; Hotta, H.; Hirata, K.; Yokoyama, M. Lysophosphatidylcholine inhibits endothelial cell migration and proliferation via inhibition of the extracellular signal-regulated kinase pathway. *Arterioscler. Thromb. Vasc. Biol.* **2000**, *20*, 1006–1012.

65. Kanda, S.; Mochizuki, Y.; Nakamura, T.; Miyata, Y.; Matsuyama, T.; Kanetake, H. Pigment epithelium-derived factor inhibits fibroblast-growth-factor-2-induced capillary morphogenesis of endothelial cells through Fyn. *J. Cell Sci.* **2005**, *118*, 961–970.

66. Sun, J.; Hopkins, B.D.; Tsujikawa, K.; Perruzzi, C.; Adini, I.; Swerlick, R.; Bornstein, P.; Lawler, J.; Benjamin, L.E. Thrombospondin-1 modulates VEGF-A-mediated Akt signaling and capillary survival in the developing retina. *Am. J. Physiol. Heart Circ. Physiol.* **2009**, *296*, H1344–H1351.

67. Zhang, J.C.; Donate, F.; Qi, X.; Ziats, N.P.; Juarez, J.C.; Mazar, A.P.; Pang, Y.P.; McCrae, K.R. The antiangiogenic activity of cleaved high molecular weight kininogen is mediated through binding to endothelial cell tropomyosin. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12224–12229.

68. Kim, J.; Cheon, I.S.; Won, Y.J.; Na, H.J.; Kim, Y.M.; Choe, J. IL-4 inhibits cell cycle progression of human umbilical vein endothelial cells by affecting p53, p21(Waf1), cyclin D1, and cyclin E expression. *Mol. Cells* **2003**, *16*, 92–96.

69. Guo, Y.L.; Wang, S.; Colman, R.W. Kininostatin, an angiogenic inhibitor, inhibits proliferation and induces apoptosis of human endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **2001**, *21*, 1427–1433.
70. Kanda, S.; Mochizuki, Y.; Miyata, Y.; Kanetake, H.; Yamamoto, N. Effects of vitamin D(3)-binding protein-derived macrophage activating factor (GcMAF) on angiogenesis. *J. Natl. Cancer Inst.* 2002, 94, 1311–1319.

71. Dixelius, J.; Larsson, H.; Sasaki, T.; Holmqvist, K.; Lu, L.; Engstrom, A.; Timpl, R.; Welsh, M.; Claesson-Welsh, L. Endostatin-induced tyrosine kinase signaling through the Shb adaptor protein regulates endothelial cell apoptosis. *Blood* 2000, 95, 3403–3411.

72. Guan, X.; Juarez, J.C.; Qi, X.; Shipulina, N.V.; Shaw, D.E.; Morgan, W.T.; McCrae, K.R.; Mazar, A.P.; Donate, F. Histidine-proline rich glycoprotein (HPRG) binds and transduces anti-angiogenic signals through cell surface tropomyosin on endothelial cells. *Thromb. Haemost.* 2004, 92, 403–412.

73. Dixelius, J.; Cross, M.; Matsumoto, T.; Sasaki, T.; Timpl, R.; Claesson-Welsh, L. Endostatin regulates endothelial cell adhesion and cytoskeletal organization. *Cancer Res.* 2002, 62, 1944–1947.

74. Rege, T.A.; Stewart, J., Jr.; Dranka, B.; Benveniste, E.N.; Silverstein, R.L.; Gladson, C.L. Thrombospondin-1-induced apoptosis of brain microvascular endothelial cells can be mediated by TNF-R1. *J. Cell Physiol.* 2009, 218, 94–103.

75. Dawson, D.W.; Volpert, O.V.; Pearce, S.F.; Schneider, A.J.; Silverstein, R.L.; Henkin, J.; Bouck, N.P. Three distinct D-amino acid substitutions confer potent antiangiogenic activity on an inactive peptide derived from a thrombospondin-1 type 1 repeat. *Mol. Pharmacol.* 1999, 55, 332–338.

76. Zhou, L.; Isenberg, J.S.; Cao, Z.; Roberts, D.D. Type I collagen is a molecular target for inhibition of angiogenesis by endogenous thrombospondin-1. *Oncogene* 2006, 25, 536-545.

77. Lafleur, M.A.; Handsley, M.M.; Knauper, V.; Murphy, G.; Ziche, M. ERK1-2 and p38 MAPK regulate MMP/TIMP balance and function in response to thrombospondin-1 fragments in the microvascular endothelium. *Life Sci.* 2004, 74, 2975–2985.

78. Staton, C.A.; Brown, N.J.; Rodgers, G.R.; Corke, K.P.; Tazzyman, S.; Underwood, J.C.; Lewis, C.E. Alphastatin, a 24-amino acid fragment of human fibrinogen, is a potent new inhibitor of activated endothelial cells in vitro and in vivo. *Blood* 2004, 103, 601–606.

79. Shellenberger, T.D.; Wang, M.; Gujratí, M.; Jayakumar, A.; Strieter, R.M.; Burdick, M.D.; Ioannides, C.G.; Efferson, C.L.; El-Naggar, A.K.; Roberts, D.; Clayman, G.L.; Frederick, M.J. BRAK/CXCL14 is a potent inhibitor of angiogenesis and a chemotactic factor for immature dendritic cells. *Cancer Res.* 2004, 64, 8262–8270.
83. Sgadari, C.; Angiolillo, A.L.; Tosato, G. Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. *Blood* **1996**, *87*, 3877–3882.

84. Angiolillo, A.L.; Sgadari, C.; Taub, D.D.; Liao, F.; Farber, J.M.; Maheshwari, S.; Kleinman, H.K.; Reaman, G.H.; Tosato, G. Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in *vivo*. *J. Exp. Med.* **1995**, *182*, 155–162.

85. Pike, S.E.; Yao, L.; Jones, K.D.; Cherney, B.; Appella, E.; Sakaguchi, K.; Nakhasi, H.; Teruya-Feldstein, J.; Wirth, P.; Gupta, G.; Tosato, G. Vasostatin, a calsiculin fragment, inhibits angiogenesis and suppresses tumor growth. *J. Exp. Med.* **1998**, *188*, 2349–2356.

86. Kaur, B.; Brat, D.J.; Devi, N.S.; Van Meir, E.G. Vasculostatin, a proteolytic fragment of Brain Angiogenesis Inhibitor 1, is an antiangiogenic and antitumorigenic factor. *Oncogene* **2005**.

87. Pepper, M.S.; Belin, D.; Montesano, R.; Orci, L.; Vassalli, J.D. Transforming growth factor-beta 1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells *in vitro*. *J. Cell Biol.* **1990**, *111*, 743–755.

88. Sato, N.; Nariuchi, H.; Tsuruoka, N.; Nishihara, T.; Beitz, J.G.; Calabresi, P.; Frackelton, A.R., Jr. Actions of TNF and IFN-gamma on angiogenesis *in vitro*. *J. Invest. Dermatol.* **1990**, *95*, 85S–89S.

89. Grant, M.B.; Caballero, S.; Millard, W.J. Inhibition of IGF-I and b-FGF stimulated growth of human retinal endothelial cells by the somatostatin analogue, octreotide: a potential treatment for ocular neovascularization. *Regul. Pept.* **1993**, *48*, 267–278.

90. Ribatti, D.; Alessandri, G.; Baronio, M.; Raffaghello, L.; Cosimo, E.; Marimpietri, D.; Montaldo, P.G.; De Falco, G.; Caruso, A.; Vacca, A.; Ponzoni, M. Inhibition of neuroblastoma-induced angiogenesis by fenretinide. *Int. J. Cancer.* **2001**, *94*, 314–321.

91. Schulter, V.; Koolwijk, P.; Peters, E.; Frank, S.; Hrzenjak, A.; Graier, W.F.; van Hinsbergh, V.W.; Kostner, G.M. Impact of apolipoprotein(a) on *in vitro* angiogenesis. *Arterioscler. Thromb. Vasc. Biol.* **2001**, *21*, 433–438.

92. Duenas, Z.; Torner, L.; Corbacho, A.M.; Ochoa, A.; Gutierrez-Ospina, G.; Lopez-Barrera, F.; Barrios, F.A.; Berger, P.; Martinez de la Escalera, G.; Clapp, C. Inhibition of rat corneal angiogenesis by 16-kDa prolactin and by endogenous prolactin-like molecules. *Invest. Ophthalmol. Vis. Sci.* **1999**, *40*, 2498–2505.

93. Russo, K.; Ragone, R.; Facchiano, A.M.; Capogrossi, M.C.; Facchiano, A. Platelet-derived growth factor-BB and basic fibroblast growth factor directly interact *in vitro* with high affinity. *J. Biol. Chem.* **2002**, *277*, 1284–1291.

94. Tucker, R.P. The thrombospondin type 1 repeat superfamily. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 969–974.

95. Karagiannis, E.D.; Popel, A.S. A systematic methodology for proteome-wide identification of peptides inhibiting the proliferation and migration of endothelial cells. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13775–13780.

96. Adams, J.C. Functions of the conserved thrombospondin carboxy-terminal cassette in cell-extracellular matrix interactions and signaling. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 1102–1114.

97. Kvansakul, M.; Adams, J.C.; Hohenester, E. Structure of a thrombospondin C-terminal fragment reveals a novel calcium core in the type 3 repeats. *Embo. J.* **2004**, *23*, 1223–1233.

98. Hogg, P.J. Thrombospondin 1 as an enzyme inhibitor. *Thromb. Haemost.* **1994**, *72*, 787–792.
99. Carlson, C.B.; Bernstein, D.A.; Annis, D.S.; Misenheimer, T.M.; Hannah, B.L.; Mosher, D.F.; Keck, J.L. Structure of the calcium-rich signature domain of human thrombospondin-2. *Nat. Struct. Mol Biol.* **2005**, *12*, 910–914.

100. Iruela-Arispe, M.L.; Lombardo, M.; Krutzsch, H.C.; Lawler, J.; Roberts, D.D. Inhibition of angiogenesis by thrombospondin-1 is mediated by 2 independent regions within the type 1 repeats. *Circulation* **1999**, *100*, 1423–1431.

101. Camozzi, M.; Rusnati, M.; Bugatti, A.; Bottazzi, B.; Mantovani, A.; Bastone, A.; Inforzato, A.; Vincenti, S.; Bracci, L.; Mastroianni, D.; Presta, M. Identification of an antiangiogenic FGF2-binding site in the N terminus of the soluble pattern recognition receptor PTX3. *J. Biol. Chem.* **2006**, *281*, 22605–22613.

102. Motegi, K.; Harada, K.; Ohe, G.; Jones, S.J.; Ellis, I.R.; Crouch, D.H.; Schor, S.L.; Schor, A.M. Differential involvement of TGF-beta1 in mediating the motogenic effects of TSP-1 on endothelial cells, fibroblasts and oral tumour cells. *Exp. Cell Res.* **2008**, *314*, 2323–2333.

103. Taraboletti, G.; Morbidelli, L.; Donnini, S.; Parenti, A.; Granger, H.J.; Giavazzi, R.; Ziche, M. The heparin binding 25 kDa fragment of thrombospondin-1 promotes angiogenesis and modulates gelatinase and TIMP-2 production in endothelial cells. *Faseb J.* **2000**, *14*, 1674–1676.

104. Ferrari do Outeiro-Bernstein, M.A.; Nunes, S.S.; Andrade, A.C.; Alves, T.R.; Legrand, C.; Morandi, V. A recombinant NH(2)-terminal heparin-binding domain of the adhesive glycoprotein, thrombospondin-1, promotes endothelial tube formation and cell survival: a possible role for syndecan-4 proteoglycan. *Matrix Biol.* **2002**, *21*, 311–324.

105. Murphy-Ullrich, J.E.; Gurusiddappa, S.; Frazer, W.A.; Hook, M. Heparin-binding peptides from thrombospondins 1 and 2 contain focal adhesion-labilizing activity. *J. Biol. Chem.* **1993**, *268*, 26784–26789.

106. Adams, J.C.; Bentley, A.A.; Kvansakul, M.; Hatherley, D.; Hohenester, E. Extracellular matrix retention of thrombospondin 1 is controlled by its conserved C-terminal region. *J. Cell Sci.* **2008**, *121*, 784–795.

107. Calzada, M.J.; Sipes, J.M.; Krutzsch, H.C.; Yurchenco, P.D.; Annis, D.S.; Mosher, D.F.; Roberts, D.D. Recognition of the N-terminal modules of thrombospondin-1 and thrombospondin-2 by alpha6beta1 integrin. *J. Biol. Chem.* **2003**, *278*, 40679–40687.

108. Vogel, T.; Guo, N.H.; Krutzsch, H.C.; Blake, D.A.; Hartman, J.; Mendelovitz, S.; Panet, A.; Roberts, D.D. Modulation of endothelial cell proliferation, adhesion, and motility by recombinant heparin-binding domain and synthetic peptides from the type I repeats of thrombospondin. *J. Cell Biochem.* **1993**, *53*, 74–84.

109. Bornstein, P. Thrombospondins function as regulators of angiogenesis. *J. Cell Commun. Signal* **2009**, *189*, 189-200.

110. Asch, A.S.; Barnwell, J.; Silverstein, R.L.; Nachman, R.L. Isolation of the thrombospondin membrane receptor. *J. Clin. Invest.* **1987**, *79*, 1054–1061.

111. Swerlick, R.A.; Lee, K.H.; Wick, T.M.; Lawley, T.J. Human dermal microvascular endothelial but not human umbilical vein endothelial cells express CD36 in vivo and in vitro. *J. Immunol.* **1992**, *148*, 78–83.
112. Dawson, D.W.; Pearce, S.F.; Zhong, R.; Silverstein, R.L.; Frazier, W.A.; Bouck, N.P. CD36 mediates the In vitro inhibitory effects of thrombospondin-1 on endothelial cells. J. Cell Biol. 1997, 138, 707–717.

113. Primo, L.; Ferrandi, C.; Roca, C.; Marchio, S.; di Blasio, L.; Alessio, M.; Bussolino, F. Identification of CD36 molecular features required for its in vitro angiostatic activity. FASEB J. 2005, 19, 1713–1715.

114. Zhang, X.; Kazeronian, S.; Duquette, M.; Perruzzi, C.; Nagy, J.A.; Dvorak, H.F.; Parangi, S.; Lawler, J. Thrombospondin-1 modulates vascular endothelial growth factor activity at the receptor level. FASEB J. 2009, 23, 3368–3376.

115. Isenberg, J.S.; Martin-Manso, G.; Maxhimer, J.B.; Roberts, D.D. Regulation of nitric oxide signalling by thrombospondin 1: implications for anti-angiogenic therapies. Nat. Rev. Cancer 2009, 9, 182–194.

116. Jimenez, B.; Volpert, O.V.; Crawford, S.E.; Febbraio, M.; Silverstein, R.L.; Bouck, N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. Nat. Med. 2000, 6, 41–48.

117. Li, K.; Yang, M.; Yuen, P.M.; Chik, K.W.; Li, C.K.; Shing, M.M.; Lam, H.K.; Fok, T.F. Thrombospondin-1 induces apoptosis in primary leukemia and cell lines mediated by CD36 and Caspase-3. Int. J. Mol. Med. 2003, 12, 995–1001.

118. Saumet, A.; Slimane, M.B.; Lanotte, M.; Lawler, J.; Dubernard, V. Type 3 repeat/C-terminal domain of thrombospondin-1 triggers caspase-independent cell death through CD47/alphavbeta3 in promyelocytic leukemia NB4 cells. Blood 2005, 106, 658–667.

119. Gao, A.G.; Lindberg, F.P.; Dimitry, J.M.; Brown, E.J.; Frazier, W.A. Thrombospondin modulates alpha v beta 3 function through integrin-associated protein. J. Cell Biol. 1996, 135, 533–544.

120. Gao, A.G.; Lindberg, F.P.; Finn, M.B.; Blystone, S.D.; Brown, E.J.; Frazier, W.A. Integrin-associated protein is a receptor for the C-terminal domain of thrombospondin. J. Biol. Chem. 1996, 271, 21–24.

121. Brown, E.J.; Frazier, W.A. Integrin-associated protein (CD47) and its ligands. Trends Cell Biol. 2001, 11, 130–135.

122. Kanda, S.; Shono, T.; Tomasin-Johansson, B.; Klint, P.; Saito, Y. Role of thrombospondin-1-derived peptide, 4N1K, in FGF-2-induced angiogenesis. Exp. Cell Res. 1999, 252, 262–272.

123. Nunes, S.S.; Outeiro-Bernstein, M.A.; Juliano, L.; Vardiero, F.; Nader, H.B.; Woods, A.; Legrand, C.; Morandi, V. Syndecan-4 contributes to endothelial tubulogenesis through interactions with two motifs inside the pro-angiogenic N-terminal domain of thrombospondin-1. J Cell Physiol. 2008, 214, 828–837.

124. Oganesian, A.; Armstrong, L.C.; Migliorini, M.M.; Strickland, D.K.; Bornstein, P. Thrombospondins use the VLDL receptor and a nonapoptotic pathway to inhibit cell division in microvascular endothelial cells. Mol. Biol. Cell 2008, 19, 563–571.

125. Orr, A.W.; Pedraza, C.E.; Pallero, M.A.; Elzie, C.A.; Goicoechea, S.; Strickland, D.K.; Murphy-Ullrich, J.E. Low density lipoprotein receptor-related protein is a calreticulin coreceptor that signals focal adhesion disassembly. J. Cell Biol. 2003, 161, 1179–1189.
126. Orr, A.W.; Elzie, C.A.; Kucik, D.F.; Murphy-Ullrich, J.E. Thrombospondin signaling through the calreticulin/LDL receptor-related protein co-complex stimulates random and directed cell migration. *J. Cell Sci.* **2003**, *116*, 2917–2927.

127. Staniszewska, I.; Zaveri, S.; Del Valle, L.; Oliva, I.; Rothman, V.L.; Croul, S.E.; Roberts, D.D.; Mosher, D.F.; Tuszynski, G.P.; Marcinkiewicz, C. Interaction of alpha9beta1 integrin with thrombospondin-1 promotes angiogenesis. *Circ. Res.* **2007**, *100*, 1308–1316.

128. Short, S.M.; Derrien, A.; Narsimhan, R.P.; Lawler, J.; Ingber, D.E.; Zetter, B.R. Inhibition of endothelial cell migration by thrombospondin-1 type-1 repeats is mediated by beta1 integrins. *J. Cell Biol.* **2005**, *168*, 643–653.

129. Chandrasekaran, L.; He, C.Z.; Al-Barazi, H.; Krutzsch, H.C.; Iruela-Arispe, M.L.; Roberts, D.D. Cell contact-dependent activation of alpha3beta1 integrin modulates endothelial cell responses to thrombospondin-1. *Mol. Biol. Cell* **2000**, *11*, 2885–2900.

130. Calzada, M.J.; Zhou, L.; Sipes, J.M.; Zhang, J.; Krutzsch, H.C.; Iruela-Arispe, M.L.; Annis, D.S.; Mosher, D.F.; Roberts, D.D. Alpha4beta1 integrin mediates selective endothelial cell responses to thrombospondins 1 and 2 in vitro and modulates angiogenesis in vivo. *Circ. Res.* **2004**, *94*, 462–470.

131. Greenaway, J.; Lawler, J.; Moorehead, R.; Bornstein, P.; Lamarre, J.; Petrik, J. Thrombospondin-1 inhibits VEGF levels in the ovary directly by binding and internalization via the low density lipoprotein receptor-related protein-1 (LRP-1). *J. Cell Physiol.* **2007**, *210*, 807–818.

132. Schultz-Cherry, S.; Chen, H.; Mosher, D.F.; Misenheimer, T.M.; Krutzsch, H.C.; Roberts, D.D.; Murphy-Ullrich, J.E. Regulation of transforming growth factor-beta activation by discrete sequences of thrombospondin 1. *J. Biol. Chem.* **1995**, *270*, 7304–7310.

133. Young, G.D.; Murphy-Ullrich, J.E. The tryptophan-rich motifs of the thrombospondin type 1 repeats bind VLAL motifs in the latent transforming growth factor-beta complex. *J. Biol. Chem.* **2004**, *279*, 47633–47642.

134. Miao, W.M.; Seng, W.L.; Duquette, M.; Lawler, P.; Laus, C.; Lawler, J. Thrombospondin-1 type 1 repeat recombinant proteins inhibit tumor growth through transforming growth factor-beta-dependent and -independent mechanisms. *Cancer Res.* **2001**, *61*, 7830–7839.

135. Hogg, P.J.; Hotchkiss, K.A.; Jimenez, B.M.; Statathakis, P.; Chesterman, C.N. Interaction of platelet-derived growth factor with thrombospondin 1. *Biochem. J.* **1997**, *326* (Pt 3), 709–716.

136. Bein, K.; Simons, M. Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. *J. Biol. Chem.* **2000**, *275*, 32167–32173.

137. Rabhi-Sabile, S.; de Romeuf, C.; Pidard, D. On the mechanism of plasmin-induced aggregation of human platelets: implication of secreted von Willebrand factor. *Thromb. Haemost.* **1998**, *79*, 1191–1198.

138. Anonick, P.K.; Yoo, J.K.; Webb, D.J.; Gonias, S.L. Characterization of the antiplasmin activity of human thrombospondin-1 in solution. *Biochem. J.* **1993**, *289* (Pt 3), 903–909.

139. Silverstein, R.L.; Leung, L.L.; Harpel, P.C.; Nachman, R.L. Complex formation of platelet thrombospondin with plasminogen. Modulation of activation by tissue activator. *J. Clin. Invest.* **1984**, *74*, 1625–1633.
140. Silverstein, R.L.; Nachman, R.L.; Leung, L.L.; Harpel, P.C. Activation of immobilized plasminogen by tissue activator. Multimolecular complex formation. *J. Biol. Chem.* **1985**, *260*, 10346–10352.

141. Silverstein, R.L.; Nachman, R.L.; Pannell, R.; Gurewich, V.; Harpel, P.C. Thrombospondin forms complexes with single-chain and two-chain forms of urokinase. *J. Biol. Chem.* **1990**, *265*, 11289–11294.

142. Hogg, P.J.; Jimenez, B.M.; Chesterman, C.N. Identification of possible inhibitory reactive centers in thrombospondin 1 that may bind cathepsin G and neutrophil elastase. *Biochemistry* **1994**, *33*, 6531–6537.

143. Hogg, P.J.; Owensby, D.A.; Chesterman, C.N. Thrombospondin 1 is a tight-binding competitive inhibitor of neutrophil cathepsin G. Determination of the kinetic mechanism of inhibition and localization of cathepsin G binding to the thrombospondin 1 type 3 repeats. *J. Biol. Chem.* **1993**, *268*, 21811–21818.

144. Mast, A.E.; Stadanlick, J.E.; Lockett, J.M.; Dietzen, D.J.; Hasty, K.A.; Hall, C.L. Tissue factor pathway inhibitor binds to platelet thrombospondin-1. *J. Biol. Chem.* **2000**, *275*, 31715–31721.

145. Feitsma, K.; Hausser, H.; Robenek, H.; Kresse, H.; Vischer, P. Interaction of thrombospondin-1 and heparan sulfate from endothelial cells. Structural requirements of heparan sulfate. *J. Biol. Chem.* **2000**, *275*, 9396–9402.

146. Panetti, T.S.; Kudryk, B.J.; Mosher, D.F. Interaction of recombinant procollagen and properdin modules of thrombospondin-1 with heparin and fibrinogen/fibrin. *J. Biol. Chem.* **1999**, *274*, 430–437.

147. Yu, H.; Tyrrell, D.; Cashel, J.; Guo, N.H.; Vogel, T.; Sipes, J.M.; Lam, L.; Fillit, H.M.; Hartman, J.; Mendelowitz, S.; Panel, A.; Roberts, D.D. Specificities of heparin-binding sites from the amino-terminus and type 1 repeats of thrombospondin-1. *Arch. Biochem. Biophys.* **2000**, *374*, 13–23.

148. Lawler, J.; Ferro, P.; Duquette, M. Expression and mutagenesis of thrombospondin. *Biochemistry* **1992**, *31*, 1173–1180.

149. Silverstein, R.L.; Leung, L.L.; Harpel, P.C.; Nachman, R.L. Platelet thrombospondin forms a trimolecular complex with plasminogen and histidine-rich glycoprotein. *J. Clin. Invest.* **1985**, *75*, 2065–2073.

150. Isordia-Salas, I.; Manns, J.M.; Sainz, I.; Parekh, H.; DeLa Cadena, R.A. Thrombospondin-1 binds to the heavy chain of elastase activated coagulation factor V (FVaHNE) and enhances thrombin generation on the surface of a promyelocytic cell line. *Thromb. Res.* **2005**, *116*, 533–543.

151. Zhou, J.; Rothman, V.L.; Sargiannidou, I.; Dimitrov, S.; Qiu, C.; Smith, E.; Sheffield, J.; Sharma, M.; Tuszynski, G.P. Cloning and characterization of angiocidin, a tumor cell binding protein for thrombospondin-1. *J. Cell Biochem.* **2004**, *92*, 125–146.

152. Hansen, G.A.; Vorum, H.; Jacobsen, C.; Honore, B. Calumenin but not reticulocalbin forms a Ca2+-dependent complex with thrombospondin-1. A potential role in haemostasis and thrombosis. *Mol. Cell Biochem.* **2009**, *320*, 25–33.

153. Faye, C.; Moreau, C.; Chautard, E.; Jetne, R.; Fukai, N.; Ruggiero, F.; Humphries, M.J.; Olsen, B.R.; Ricard-Blum, S. Molecular interplay between endostatin, integrins, and heparan sulfate. *J. Biol. Chem.* **2009**, *284*, 22029–22040.
154. Floquet, N.; Dedieu, S.; Martiny, L.; Dauchez, M.; Perahia, D. Human thrombospondin's (TSP-1) C-terminal domain opens to interact with the CD-47 receptor: a molecular modeling study. *Arch. Biochem. Biophys.* 2008, 478, 103–109.

155. Elzie, C.A.; Murphy-Ullrich, J.E. The N-terminus of thrombospondin: the domain stands apart. *Int. J. Biochem. Cell Biol.* 2004, 36, 1090–1101.

156. Mikhailenko, I.; Krylov, D.; Argraves, K.M.; Roberts, D.D.; Liau, G.; Strickland, D.K. Cellular internalization and degradation of thrombospondin-1 is mediated by the amino-terminal heparin binding domain (HBD). High affinity interaction of dimeric HBD with the low density lipoprotein receptor-related protein. *J. Biol. Chem.* 1997, 272, 6784–6791.

157. Tan, K.; Duquette, M.; Liu, J.H.; Zhang, R.; Joachimiak, A.; Wang, J.H.; Lawler, J. The structures of the thrombospondin-1 N-terminal domain and its complex with a synthetic pentameric heparin. *Structure* 2006, 14, 33–42.

158. Michalak, M.; Corbett, E.F.; Mesaeli, N.; Nakamura, K.; Opas, M. Calreticulin: one protein, one gene, many functions. *Biochem. J.* 1999, 344 (Pt 2), 281–292.

159. Li, Z.; Calzada, M.J.; Sipes, J.M.; Cashel, J.A.; Krutzsch, H.C.; Annis, D.S.; Mosher, D.F.; Roberts, D.D. Interactions of thrombospondins with alpha4beta1 integrin and CD47 differentially modulate T cell behavior. *J. Cell Biol.* 2002, 157, 509–519.

160. Calzada, M.J.; Roberts, D.D. Novel integrin antagonists derived from thrombospondins. *Curr. Pharm. Des.* 2005, 11, 849–866.

161. Furrer, J.; Luy, B.; Basrur, V.; Roberts, D.D.; Barchi, J.J., Jr. Conformational analysis of an alpha3beta1 integrin-binding peptide from thrombospondin-1: implications for antiangiogenic drug design. *J. Med. Chem.* 2006, 49, 6324–6333.

162. Krutzsch, H.C.; Choe, B.J.; Sipes, J.M.; Guo, N.; Roberts, D.D. Identification of an alpha(3)beta(1) integrin recognition sequence in thrombospondin-1. *J. Biol. Chem.* 1999, 274, 24080–24086.

163. Calzada, M.J.; Annis, D.S.; Zeng, B.; Marcinkiewicz, C.; Banas, B.; Lawler, J.; Mosher, D.F.; Roberts, D.D. Identification of novel beta1 integrin binding sites in the type 1 and type 2 repeats of thrombospondin-1. *J. Biol. Chem.* 2004, 279, 41734–41743.

164. Lahav, J.; Schwartz, M.A.; Hynes, R.O. Analysis of platelet adhesion with a radioactive chemical crosslinking reagent: interaction of thrombospondin with fibronectin and collagen. *Cell* 1982, 31, 253–262.

165. Mumby, S.M.; Raugi, G.J.; Bornstein, P. Interactions of thrombospondin with extracellular matrix proteins: selective binding to type V collagen. *J. Cell Biol.* 1984, 98, 646–652.

166. Galvin, N.J.; Vance, P.M.; Dixit, V.M.; Fink, B.; Frazier, W.A. Interaction of human thrombospondin with types I-V collagen: direct binding and electron microscopy. *J. Cell Biol.* 1987, 104, 1413–1422.

167. Sottile, J.; Hocking, D.C. Fibronectin polymerization regulates the composition and stability of extracellular matrix fibrils and cell-matrix adhesions. *Mol. Biol. Cell* 2002, 13, 3546–3559.

168. Lahav, J.; Lawler, J.; Gimbrone, M.A. Thrombospondin interactions with fibronectin and fibrinogen. Mutual inhibition in binding. *Eur. J. Biochem.* 1984, 145, 151–156.

169. Bale, M.D.; Mosher, D.F. Thrombospondin is a substrate for blood coagulation factor XIIIa. *Biochemistry* 1986, 25, 5667–5673.
170. Bale, M.D.; Mosher, D.F. Effects of thrombospondin on fibrin polymerization and structure. *J. Biol. Chem.* 1986, 261, 862–868.

171. Pimanda, J.E.; Annis, D.S.; Raftery, M.; Mosher, D.F.; Chesterman, C.N.; Hogg, P.J. The von Willebrand factor-reducing activity of thrombospondin-1 is located in the calcium-binding/C-terminal sequence and requires a free thiol at position 974. *Blood* 2002, 100, 2832–2838.

172. Merle, B.; Malaval, L.; Lawler, J.; Delmas, P.; Clezardin, P. Decorin inhibits cell attachment to thrombospondin-1 by binding to a KKTR-dependent cell adhesive site present within the N-terminal domain of thrombospondin-1. *J. Cell Biochem.* 1997, 67, 75–83.

173. Morales, A.M.; Maile, L.A.; Clarke, J.; Busby, W.H., Jr.; Clemmons, D.R. Insulin-like growth factor binding protein-5 (IGFBP-5) interacts with thrombospondin-1 to induce negative regulatory effects on IGF-I actions. *J. Cell Physiol.* 2005, 203, 328–334.

174. Taraboletti, G.; Benelli, R.; Borsotti, P.; Rusnati, M.; Presta, M.; Giavazzi, R.; Ruco, L.; Albini, A. Thrombospondin-1 inhibits Kaposi's sarcoma (KS) cell and HIV-1 Tat-induced angiogenesis and is poorly expressed in KS lesions. *J. Pathol.* 1999, 188, 76–81.

175. Yang, Z.; Strickland, D.K.; Bornstein, P. Extracellular matrix metalloproteinase 2 levels are regulated by the low density lipoprotein-related scavenger receptor and thrombospondin 2. *J. Biol. Chem.* 2001, 276, 8403–8408.

176. Iruela-Arispe, M.L.; Luque, A.; Lee, N. Thrombospondin modules and angiogenesis. *Int. J. Biochem. Cell Biol.* 2004, 36, 1070–1078.

177. Rodriguez-Manzaneque, J.C.; Lane, T.F.; Ortega, M.A.; Hynes, R.O.; Lawler, J.; Iruela-Arispe, M.L. Thrombospondin-1 suppresses spontaneous tumor growth and inhibits activation of matrix metalloproteinase-9 and mobilization of vascular endothelial growth factor. *Proc. Natl. Acad. Sci. USA* 2001, 98, 12485–12490.

178. Qian, X.; Wang, T.N.; Rothman, V.L.; Nicosia, R.F.; Tuszynski, G.P. Thrombospondin-1 modulates angiogenesis in vitro by up-regulation of matrix metalloproteinase-9 in endothelial cells. *Exp. Cell Res.* 1997, 235, 403–412.

179. Wang, S.; Skorczewski, J.; Feng, X.; Mei, L.; Murphy-Ullrich, J.E. Glucose up-regulates thrombospondin 1 gene transcription and transforming growth factor-beta activity through antagonism of cGMP-dependent protein kinase repression via upstream stimulatory factor 2. *J. Biol. Chem.* 2004, 279, 34311–34322.

180. Panigrahy, D.; Kaipainen, A.; Huang, S.; Butterfield, C.E.; Barnes, C.M.; Fannon, M.; Laforme, A.M.; Chaponis, D.M.; Folkman, J.; Kieran, M.W. PPARalpha agonist fenofibrate suppresses tumor growth through direct and indirect angiogenesis inhibition. *Proc. Natl. Acad. Sci. USA* 2008, 105, 985–990.

181. Kang, J.H.; Kim, S.A.; Chang, S.Y.; Hong, S.; Hong, K.J. Inhibition of trichostatin A-induced antiangiogenesis by small-interfering RNA for thrombospondin-1. *Exp. Mol. Med.* 2007, 39, 402–411.

182. Castle, V.P.; Ou, X.; O'Shea, S.; Dixit, V.M. Induction of thrombospondin 1 by retinoic acid is important during differentiation of neuroblastoma cells. *J. Clin. Invest.* 1992, 90, 1857–1863.

183. Kim, S.A.; Kang, J.H.; Cho, I.; Bae, S.W.; Hong, K.J. Cell-type specific regulation of thrombospondin-1 expression and its promoter activity by regulatory agents. *Exp. Mol. Med.* 2001, 33, 117–123.
184. Doebele, R.C.; Schulze-Hoepfner, F.T.; Hong, J.; Chlenski, A.; Zeitlin, B.D.; Goel, K.; Gomes, S.; Liu, Y.; Abe, M.K.; Nor, J.E.; Lingen, M.W.; Rosner, M.R. A novel interplay between Epac/Rap1 and mitogen-activated protein kinase kinase 5/extracellular signal-regulated kinase 5 (MEK5/ERK5) regulates thrombospondin to control angiogenesis. Blood 2009, 114, 4592–4600.

185. Lee, T.Y.; Muschal, S.; Pravda, E.A.; Folkman, J.; Abdollahi, A.; Javaherian, K. Angiostatin regulates the expression of antiangiogenic and proapoptotic pathways via targeted inhibition of mitochondrial proteins. Blood 2009, 114, 1987–1998.

186. Puri, N.; Khramtsov, A.; Ahmed, S.; Nallasura, V.; Hetzel, J.T.; Jagadeeswaran, R.; Karczmar, G.; Salgia, R. A selective small molecule inhibitor of c-Met, PHA665752, inhibits tumorigenicity and angiogenesis in mouse lung cancer xenografts. Cancer Res. 2007, 67, 3529–3534.

187. Mirkin, S.; Mahony, M.C.; Archer, D.F. Effect of tibolone and its metabolites on vascular endothelial growth factor isoforms 121 and 165 and thrombospondin-1 mRNA in Ishikawa cells. Menopause 2004, 11, 82–88.

188. Albig, A.R.; Schiemann, W.P. Fibulin-5 antagonizes vascular endothelial growth factor (VEGF) signaling and angiogenic sprouting by endothelial cells. DNA Cell Biol. 2004, 23, 367–379.

189. Naito, T.; Masaki, T.; Nikolic-Paterson, D.J.; Tanji, C.; Yorioka, N.; Kohno, N. Angiotensin II induces thrombospondin-1 production in human mesangial cells via p38 MAPK and JNK: a mechanism for activation of latent TGF-beta1. Am. J. Physiol. Renal Physiol. 2004, 286, F278-F287.

190. Fischer, J.W.; Stoll, M.; Hahn, A.W.; Unger, T. Differential regulation of thrombospondin-1 and fibronectin by angiotensin II receptor subtypes in cultured endothelial cells. Cardiovase. Res. 2001, 51, 784–791.

191. Ding, I.; Sun, J.Z.; Fenton, B.; Liu, W.M.; Kimsely, P.; Okunieff, P.; Min, W. Intratumoral administration of endostatin plasmid inhibits vascular growth and perfusion in MCa-4 murine mammary carcinomas. Cancer Res. 2001, 61, 526–531.

192. Hyder, S.M.; Liang, Y.; Wu, J. Estrogen regulation of thrombospondin-1 in human breast cancer cells. Int. J. Cancer. 2009, 125, 1045–1053.

193. Navarro, F.J.; Mirkin, S.; Archer, D.F. Effect of raloxifene, 17beta-estradiol, and progesterone on mRNA for vascular endothelial growth factor isoforms 121 and 165 and thrombospondin-1 in Ishikawa cells. Fertil. Steril. 2003, 79, 1409–1415.

194. Kim, J.; Kim, C.; Kim, T.S.; Bang, S.I.; Yang, Y.; Park, H.; Cho, D. IL-18 enhances thrombospondin-1 production in human gastric cancer via JNK pathway. Biochem. Biophys. Res. Commun. 2006, 344, 1284–1289.

195. Congote, L.F.; DiFalco, M.R.; Gibbs, B.F. Thrombospondin 1, produced by endothelial cells under the action of erythropoietin, stimulates thymidine incorporation into erythroid cells and counteracts the inhibitory action of insulin-like growth factor binding protein 3. Cytokine 2005, 30, 248–253.

196. Soula-Rothhut, M.; Coissard, C.; Sartelet, H.; Boudot, C.; Bellon, G.; Martiny, L.; Rothhut, B. The tumor suppressor PTEN inhibits EGF-induced TSP-1 and TIMP-1 expression in FTC-133 thyroid carcinoma cells. Exp. Cell Res. 2005, 304, 187–201.
197. Horiguchi, H.; Jin, L.; Ruebel, K.H.; Scheithauer, B.W.; Lloyd, R.V. Regulation of VEGF-A, VEGFR-I, thrombospondin-1, -2, and -3 expression in a human pituitary cell line (HP75) by TGFbeta1, bFGF, and EGF. *Endocrine* 2004, 24, 141–146.

198. Martinez-Sales, V.; Vila, V.; Ferrando, M.; Reganou, E. Atorvastatin neutralizes the up-regulation of thrombospondin-1 induced by thrombin in human umbilical vein endothelial cells. *Endothelium* 2007, 14, 233–238.

199. Hellebrekers, D.M.; Jair, K.W.; Vire, E.; Eguchi, S.; Hoebers, N.T.; Fraga, M.F.; Esteller, M.; Fuks, F.; Baylin, S.B.; van Engeland, M.; Griffioen, A.W. Angiostatic activity of DNA methyltransferase inhibitors. *Mol. Cancer Ther.* 2006, 5, 467–475.

200. Liu, Z.; Christensson, M.; Forslow, A.; De Meester, I.; Sundqvist, K.G. A CD26-controlled cell surface cascade for regulation of T cell motility and chemokine signals. *J. Immunol.* 2009, 183, 3616–3624.

201. Bocci, G.; Francia, G.; Man, S.; Lawler, J.; Kerbel, R.S. Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. *Proc. Natl. Acad. Sci. USA* 2003, 100, 12917–12922.

202. Hamano, Y.; Sugimoto, H.; Soubasakos, M.A.; Kieran, M.; Olsen, B.R.; Lawler, J.; Sudhakar, A.; Kalluri, R. Thrombospondin-1 associated with tumor microenvironment contributes to low-dose cyclophosphamide-mediated endothelial cell apoptosis and tumor growth suppression. *Cancer Res.* 2004, 64, 1570–1574.

203. Zhao, H.Y.; Ooyama, A.; Yamamoto, M.; Ikeda, R.; Haraguchi, M.; Tabata, S.; Furukawa, T.; Che, X.F.; Ishiwata, K.; Oka, T.; Fukushima, M.; Nakagawa, M.; Ono, M.; Kuwano, M.; Akiyama, S. Down regulation of c-Myc and induction of an angiogenesis inhibitor, thrombospondin-1, by 5-FU in human colon cancer KM12C cells. *Cancer Lett.* 2008, 270, 156–163.

204. Damber, J.E.; Vallbo, C.; Albertsson, P.; Lennernas, B.; Norrby, K. The anti-tumour effect of low-dose continuous chemotherapy may partly be mediated by thrombospondin. *Cancer Chemother. Pharmacol.* 2006, 58, 354–360.

205. Yoo, G.H.; Piechocki, M.P.; Ensley, J.F.; Nguyen, T.; Oliver, J.; Meng, H.; Kewson, D.; Shibuya, T.Y.; Lonardo, F.; Tainsky, M.A. Docetaxel induced gene expression patterns in head and neck squamous cell carcinoma using cDNA microarray and PowerBlot. *Clin. Cancer Res.* 2002, 8, 3910–3921.

206. Cinatl, J., Jr.; Kotchetkov, R.; Blaheta, R.; Driever, P.H.; Vogel, J.U.; Cinatl, J. Induction of differentiation and suppression of malignant phenotype of human neuroblastoma BE(2)-C cells by valproic acid: enhancement by combination with interferon-alpha. *Int. J. Oncol.* 2002, 20, 97–106.

207. Bocci, G.; Falcone, A.; Fioravanti, A.; Orlandi, P.; Di Paolo, A.; Fanelli, G.; Viacava, P.; Naccarato, A.G.; Kerbel, R.S.; Danesi, R.; Del Tacca, M.; Allegrini, G. Antiangiogenic and anticolorectal cancer effects of metronomic irinotecan chemotherapy alone and in combination with semaxinib. *Br. J. Cancer.* 2008, 98, 1619–1629.

208. Allegrini, G.; Falcone, A.; Fioravanti, A.; Barletta, M.T.; Orlandi, P.; Loupakis, F.; Cerri, E.; Masi, G.; Di Paolo, A.; Kerbel, R.S.; Danesi, R.; Del Tacca, M.; Bocci, G. A pharmacokinetic
and pharmacodynamic study on metronomic irinotecan in metastatic colorectal cancer patients. *Br. J. Cancer.* **2008**, *98*, 1312–1319.

209. Castle, V.P.; Dixit, V.M.; Polverini, P.J. Thrombospondin-1 suppresses tumorigenesis and angiogenesis in serum- and anchorage-independent NIH 3T3 cells. *Lab. Invest.* **1997**, *77*, 51–61.

210. Zhang, X.; Xu, J.; Lawler, J.; Terwilliger, E.; Parangi, S. Adeno-associated virus-mediated antiangiogenic gene therapy with thrombospondin-1 type 1 repeats and endostatin. *Clin. Cancer Res.* **2007**, *13*, 3968–3976.

211. Liu, P.; Wang, Y.; Li, Y.H.; Yang, C.; Zhou, Y.L.; Li, B.; Lu, S.H.; Yang, R.C.; Cai, Y.L.; Tobelem, G.; Caen, J.; Han, Z.C. Adenovirus-mediated gene therapy with an antiangiogenic fragment of thrombospondin-1 inhibits human leukemia xenograft growth in nude mice. *Leuk. Res.* **2003**, *27*, 701–708.

212. Yee, K.O.; Streit, M.; Hawighorst, T.; Detmar, M.; Lawler, J. Expression of the type-1 repeats of thrombospondin-1 inhibits tumor growth through activation of transforming growth factor-beta. *Am. J. Pathol.* **2004**, *165*, 541–552.

213. Miyata, Y.; Koga, S.; Takehara, K.; Kanetake, H.; Kanda, S. Expression of thrombospondin-derived 4N1K peptide-containing proteins in renal cell carcinoma tissues is associated with a decrease in tumor growth and angiogenesis. *Clin. Cancer Res.* **2003**, *9*, 1734–1740.

214. Amagasaki, K.; Sasaki, A.; Kato, G.; Maeda, S.; Nukui, H.; Naganuma, H. Antisense-mediated reduction in thrombospondin-1 expression reduces cell motility in malignant glioma cells. *Int. J. Cancer.* **2001**, *94*, 508–512.

215. Dameron, K.M.; Volpert, O.V.; Tainsky, M.A.; Bouck, N. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* **1994**, *265*, 1582–1584.

216. Dameron, K.M.; Volpert, O.V.; Tainsky, M.A.; Bouck, N. The p53 tumor suppressor gene inhibits angiogenesis by stimulating the production of thrombospondin. *Cold Spring Harb. Symp. Quant. Biol.* **1994**, *59*, 483–489.

217. Gautam, A.; Densmore, C.L.; Melton, S.; Golunski, E.; Waldrep, J.C. Aerosol delivery of PEI-p53 complexes inhibits B16-F10 lung metastases through regulation of angiogenesis. *Cancer Gene Ther.* **2002**, *9*, 28–36.

218. Suarez, Y.; Fernandez-Hernando, C.; Yu, J.; Gerber, S.A.; Harrison, K.D.; Pober, J.S.; Iruela-Arispe, M.L.; Merkenschlager, M.; Sessa, W.C. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14082–14087.

219. Dews, M.; Homayouni, A.; Yu, D.; Murphy, D.; Sevignani, C.; Wentzel, E.; Furth, E.E.; Lee, W.M.; Enders, G.H.; Mendell, J.T.; Thomas-Tikhonenko, A. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat. Genet.* **2006**, *38*, 1060–1065.

220. Qin, H.; Shao, Q.; Thomas, T.; Kalra, J.; Alaoui-Jamali, M.A.; Laird, D.W. Connexin26 regulates the expression of angiogenesis-related genes in human breast tumor cells by both GJIC-dependent and -independent mechanisms. *Cell Commun. Adhes.* **2003**, *10*, 387–393.

221. Yang, G.; Cai, K.Q.; Thompson-Lanza, J.A.; Bast, R.C., Jr.; Liu, J. Inhibition of breast and ovarian tumor growth through multiple signaling pathways by using retrovirus-mediated small interfering RNA against Her-2/neu gene expression. *J. Biol. Chem.* **2004**, *279*, 4339–4345.
222. de Fraipont, F.; Keramidas, M.; El Atifi, M.; Chambaz, E.M.; Berger, F.; Feige, J.J. Expression of the thrombospondin 1 fragment 167-569 in C6 glioma cells stimulates tumorigenicity despite reduced neovascularization. *Oncogene* 2004, 23, 3642–3649.

223. Wells, J.A.; McClendon, C.L. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature* 2007, 450, 1001–1009.

224. Robinson, J.A.; Demarco, S.; Gombert, F.; Moehle, K.; Obrecht, D. The design, structures and therapeutic potential of protein epitope mimetics. *Drug Discov. Today* 2008, 13, 944–951.

225. Murray, J.K.; Gellman, S.H. Targeting protein-protein interactions: lessons from p53/MDM2. *Biopolymers* 2007, 88, 657–686.

226. Sulochana, K.N.; Ge, R. Developing antiangiogenic peptide drugs for angiogenesis-related diseases. *Curr. Pharm. Des.* 2007, 13, 2074–2086.

227. Tolsma, S.S.; Volpert, O.V.; Good, D.J.; Frazier, W.A.; Polverini, P.J.; Bouck, N. Peptides derived from two separate domains of the matrix protein thrombospondin-1 have anti-angiogenic activity. *J. Cell Biol.* 1993, 122, 497–511.

228. Lee, N.V.; Sato, M.; Annis, D.S.; Luo, J.A.; Wu, L.; Mosher, D.F.; Iruela-Arispe, M.L. ADAMTS1 mediates the release of antiangiogenic polypeptides from TSP1 and 2. *Embo. J.* 2006, 25, 5270–5283.

229. Brul, A.; Touhami-Carrier, M.; Thomaidis, A.; Legrand, C. Thrombospondin-1 (TSP-1) and TSP-1-derived heparin-binding peptides induce promyelocytic leukemia cell differentiation and apoptosis. *Anticancer Res.* 2005, 25, 757–764.

230. Zhang, X.; Galardi, E.; Duquette, M.; Delic, M.; Lawler, J.; Parangi, S. Antiangiogenic treatment with the three thrombospondin-1 type 1 repeats recombinant protein in an orthotopic human pancreatic cancer model. *Clin. Cancer Res.* 2005, 11, 2337–2344.

231. Zhang, X.; Connolly, C.; Duquette, M.; Lawler, J.; Parangi, S. Continuous administration of the three thrombospondin-1 type 1 repeats recombinant protein improves the potency of therapy in an orthotopic human pancreatic cancer model. *Cancer Lett.* 2007, 247, 143–149.

232. Yee, K.O.; Connolly, C.M.; Duquette, M.; Kazerounian, S.; Washington, R.; Lawler, J. The effect of thrombospondin-1 on breast cancer metastasis. *Breast Cancer Res. Treat.* 2009, 114, 85–96.

233. Guo, N.H.; Krutzsch, H.C.; Inman, J.K.; Shannon, C.S.; Roberts, D.D. Antiproliferative and antitumor activities of D-reverse peptides derived from the second type-1 repeat of thrombospondin-1. *J. Pept. Res.* 1997, 50, 210–221.

234. Jimenez, B.; Volpert, O.V.; Reiher, F.; Chang, L.; Munoz, A.; Karin, M.; Bouck, N. c-Jun N-terminal kinase activation is required for the inhibition of neovascularization by thrombospondin-1. *Oncogene* 2001, 20, 3443–3448.

235. Guo, N.; Krutzsch, H.C.; Inman, J.K.; Roberts, D.D. Thrombospondin 1 and type I repeat peptides of thrombospondin 1 specifically induce apoptosis of endothelial cells. *Cancer Res.* 1997, 57, 1735–1742.

236. Manna, P.P.; Frazier, W.A. CD47 mediates killing of breast tumor cells via Gi-dependent inhibition of protein kinase A. *Cancer Res.* 2004, 64, 1026–1036.

237. Lih, C.J.; Wei, W.; Cohen, S.N. Txr1: a transcriptional regulator of thrombospondin-1 that modulates cellular sensitivity to taxanes. *Genes Dev.* 2006, 20, 2082–2095.
238. Rath, G.M.; Schneider, C.; Dedieu, S.; Sartelet, H.; Morjani, H.; Martiny, L.; El Btaouri, H. Thrombospondin-1 C-terminal-derived peptide protects thyroid cells from ceramide-induced apoptosis through the adenylyl cyclase pathway. *Int. J. Biochem. Cell Biol.* **2006**, *38*, 2219–2228.

239. Goldfarb, D.S.; Gariepy, J.; Schoolnik, G.; Kornberg, R.D. Synthetic peptides as nuclear localization signals. *Nature* **1986**, *322*, 641–644.

240. Nestor, J.J., Jr. The medicinal chemistry of peptides. *Curr Med Chem.* **2009**, *16*, 4399–4418.

241. Bogdanov, A., Jr.; Marcos, E.; Cheng, H.C.; Chandrasekaran, L.; Krutzsch, H.C.; Roberts, D.D.; Weissleder, R. Treatment of experimental brain tumors with thrombospondin-1 derived peptides: an *in vivo* imaging study. *Neoplasia* **1999**, *1*, 438–445.

242. Coronella, J.; Li, L.; Johnson, K.; Pirie-Shepherd, S.; Roxas, G.; Levin, N. Selective activity against proliferating tumor endothelial cells by CVX-22, a thrombospondin-1 mimetic CovX-Body. *Anticancer Res.* **2009**, *29*, 2243–2252.

243. Molckovsky, A.; Siu, L.L. First-in-class, first-in-human phase I results of targeted agents: Highlights of the 2008 American Society of Clinical Oncology meeting. *J. Hematol. Oncol.* **2008**, *1*, 20.

244. Reiher, F.K.; Volpert, O.V.; Jimenez, B.; Crawford, S.E.; Dinney, C.P.; Henkin, J.; Haviv, F.; Bouck, N.P.; Campbell, S.C. Inhibition of tumor growth by systemic treatment with thrombospondin-1 peptide mimetics. *Int. J. Cancer.* **2002**, *98*, 682–689.

245. Haviv, F.; Bradley, M.F.; Kalvin, D.M.; Schneider, A.J.; Davidson, D.J.; Majest, S.M.; McKay, L.M.; Haskell, C.J.; Bell, R.L.; Nguyen, B.; Marsh, K.C.; Surber, B.W.; Uchic, J.T.; Ferrero, J.; Wang, Y.C.; Leal, J.; Record, R.D.; Hodde, J.; Badylak, S.F.; Lesniewski, R.R.; Henkin, J. Thrombospondin-1 mimetic peptide inhibitors of angiogenesis and tumor growth: design, synthesis, and optimization of pharmacokinetics and biological activities. *J. Med. Chem.* **2005**, *48*, 2838–2846.

246. Greenaway, J.; Henkin, J.; Lawler, J.; Moorehead, R.; Petrik, J. ABT-510 induces tumor cell apoptosis and inhibits ovarian tumor growth in an orthotopic, syngeneic model of epithelial ovarian cancer. *Mol. Cancer Ther.* **2009**, *8*, 64–74.

247. Anderson, J.C.; Grammer, J.R.; Wang, W.; Nabors, L.B.; Henkin, J.; Stewart, J.E., Jr.; Gladson, C.L. ABT-510, a modified type 1 repeat peptide of thrombospondin, inhibits malignant glioma growth *in vivo* by inhibiting angiogenesis. *Cancer Biol. Ther.* **2007**, *6*, 454–462.

248. Quesada, A.J.; Nelius, T.; Yap, R.; Zaichuk, T.A.; Alfranca, A.; Filleur, S.; Volpert, O.V.; Redondo, J.M. *In vivo* upregulation of CD95 and CD95L causes synergistic inhibition of angiogenesis by TSP1 peptide and metronomic doxorubicin treatment. *Cell Death Differ.* **2005**, *12*, 649–658.

249. Viloria-Petit, A.; Miquerol, L.; Yu, J.L.; Gertsenstein, M.; Sheehan, C.; May, L.; Henkin, J.; Lobe, C.; Nagy, A.; Kerbel, R.S.; Rak, J. Contrasting effects of VEGF gene disruption in embryonic stem cell-derived *versus* oncogene-induced tumors. *EMBO J.* **2003**, *22*, 4091–4102.

250. Rusk, A.; Cozzi, E.; Stebbins, M.; Vail, D.; Graham, J.; Valli, V.; Henkin, J.; Sharpee, R.; Khanna, C. Cooperative activity of cytotoxic chemotherapy with antiangiogenic thrombospondin-1 peptides, ABT-526 in pet dogs with relapsed lymphoma. *Clin. Cancer Res.* **2006**, *12*, 7456–7464.
251. Hoekstra, R.; de Vos, F.Y.; Eskens, F.A.; Gietema, J.A.; van der Gaast, A.; Groen, H.J.; Knight, R.A.; Carr, R.A.; Humerickhouse, R.A.; Verweij, J.; de Vries, E.G. Phase I safety, pharmacokinetic, and pharmacodynamic study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 in patients with advanced cancer. *J. Clin. Oncol.* **2005**, *23*, 5188–5197.

252. Baker, L.H.; Rowinsky, E.K.; Mendelson, D.; Humerickhouse, R.A.; Knight, R.A.; Qian, J.; Carr, R.A.; Gordon, G.B.; Demetri, G.D. Randomized, phase II study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 in patients with advanced soft tissue sarcoma. *J. Clin. Oncol.* **2008**, *26*, 5583–5588.

253. Gordon, M.S.; Mendelson, D.; Carr, R.; Knight, R.A.; Humerickhouse, R.A.; Iannone, M.; Stopeck, A.T. A phase I trial of 2 dose schedules of ABT-510, an antiangiogenic, thrombospondin-1-mimetic peptide, in patients with advanced cancer. *Cancer* **2008**, *113*, 3420–3429.

254. Ebbinghaus, S.; Hussain, M.; Tannir, N.; Gordon, M.; Desai, A.A.; Knight, R.A.; Humerickhouse, R.A.; Qian, J.; Gordon, G.B.; Figlin, R. Phase 2 study of ABT-510 in patients with previously untreated advanced renal cell carcinoma. *Clin. Cancer Res.* **2007**, *13*, 6689–6695.

255. Markovic, S.N.; Suman, V.J.; Rao, R.A.; Ingle, J.N.; Kaur, J.S.; Erickson, L.A.; Pitot, H.C.; Crogan, G.A.; McWilliams, R.R.; Merchan, J.; Kottschade, L.A.; Nevala, W.K.; Uhl, C.B.; Allred, J.; Creagan, E.T. A phase II study of ABT-510 (thrombospondin-1 analog) for the treatment of metastatic melanoma. *Am. J. Clin. Oncol.* **2007**, *30*, 303–309.

256. Yang, Q.; Tian, Y.; Liu, S.; Zeine, R.; Chlenski, A.; Salwen, H.R.; Henkin, J.; Cohn, S.L. Thrombospondin-1 peptide ABT-510 combined with valproic acid is an effective antiangiogenesis strategy in neuroblastoma. *Cancer Res.* **2007**, *67*, 1716–1724.

257. Gietema, J.A.; Hoekstra, R.; de Vos, F.Y.; Uges, D.R.; van der Gaast, A.; Groen, H.J.; Loos, W.J.; Knight, R.A.; Carr, R.A.; Humerickhouse, R.A.; Eskens, F.A. A phase I study assessing the safety and pharmacokinetics of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 with gemcitabine and cisplatin in patients with solid tumors. *Ann. Oncol.* **2006**, *17*, 1320–1327.

258. Hoekstra, R.; de Vos, F.Y.; Eskens, F.A.; de Vries, E.G.; Uges, D.R.; Knight, R.; Carr, R.A.; Humerickhouse, R.; Verweij, J.; Gietema, J.A. Phase I study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 with 5-fluorouracil and leucovorin: a safe combination. *Eur. J. Cancer* **2006**, *42*, 467–472.

259. Clackson, T.; Wells, J.A. A hot spot of binding energy in a hormone-receptor interface. *Science* **1995**, *267*, 383–386.

260. Thanos, C.D.; DeLano, W.L.; Wells, J.A. Hot-spot mimicry of a cytokine receptor by a small molecule. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15422–15427.

261. Martin-Manso, G.; Galli, S.; Ridnour, L.A.; Tsokos, M.; Wink, D.A.; Roberts, D.D. Thrombospondin 1 promotes tumor macrophage recruitment and enhances tumor cell cytotoxicity of differentiated U937 cells. *Cancer Res.* **2008**, *68*, 7090–7099.

262. Yang, M.; Li, K.; Ng, M.H.; Yuen, P.M.; Fok, T.F.; Li, C.K.; Hogg, P.J.; Chong, B.H. Thrombospondin-1 inhibits *in vitro* megakaryocytopoiesis via CD36. *Thromb. Res.* **2003**, *109*, 47–54.

263. Castelli, R.; Porro, F.; Tarsia, P. The heparins and cancer: review of clinical trials and biological properties. *Vasc. Med.* **2004**, *9*, 205–213.
264. John, A.S.; Hu, X.; Rothman, V.L.; Tuszyński, G.P. Thrombospondin-1 (TSP-1) up-regulates tissue inhibitor of metalloproteinase-1 (TIMP-1) production in human tumor cells: exploring the functional significance in tumor cell invasion. Exp. Mol. Pathol. 2009, 87, 184–188.

265. Kang, S.Y.; Halvorsen, O.J.; Gravdal, K.; Bhattacharya, N.; Lee, J.M.; Liu, N.W.; Johnston, B.T.; Johnston, A.B.; Haukaas, S.A.; Aamodt, K.; Yoo, S.; Akslen, L.A.; Watnick, R.S. Prosaposin inhibits tumor metastasis via paracrine and endocrine stimulation of stromal p53 and Tsp-1. Proc. Natl. Acad. Sci. USA 2009, 106, 12115–12120.

266. Koskimaki, J.E.; Karagiannis, E.D.; Rosca, E.V.; Vesuna, F.; Winnard, P.T., Jr.; Raman, V.; Bhujwala, Z.M.; Popel, A.S. Peptides derived from type IV collagen, CXC chemokines, and thrombospondin-1 domain-containing proteins inhibit neovascularization and suppress tumor growth in MDA-MB-231 breast cancer xenografts. Neoplasia 2009, 11, 1285–1291.

267. Li, Z.; He, L.; Wilson, K.; Roberts, D. Thrombospondin-1 inhibits TCR-mediated T lymphocyte early activation. J. Immunol. 2001, 166, 2427–2436.

268. Johansson, U.; Higginbottom, K.; Londei, M. CD47 ligation induces a rapid caspase-independent apoptosis-like cell death in human monocytes and dendritic cells. Scand. J. Immunol. 2004, 59, 40–49.

269. Turpie, B.; Yoshimura, T.; Gulati, A.; Rios, J.D.; Dartt, D.A.; Masli, S. Sjogren's syndrome-like ocular surface disease in thrombospondin-1 deficient mice. Am. J. Pathol. 2009, 175, 1136–1147.

270. Yang, K.; Vega, J.L.; Hadzipasic, M.; Schatzmann Peron, J.P.; Zhu, B.; Carrier, Y.; Masli, S.; Rizzo, L.V.; Weiner, H.L. Deficiency of thrombospondin-1 reduces Th17 differentiation and attenuates autoimmune encephalomyelitis. J. Autoimmun. 2009, 32, 94–103.

271. Tabib, A.; Krispin, A.; Trahtemberg, U.; Verbovetski, I.; Lebendiker, M.; Danieli, T.; Mevorach, D. Thrombospondin-1-N-terminal domain induces a phagocytic state and thrombospondin-1-C-terminal domain induces a tolerizing phenotype in dendritic cells. PLoS One 2009, 4, e6840.

272. Yamauchi, Y.; Kuroki, M.; Imakiire, T.; Uno, K.; Abe, H.; Beppu, R.; Yamashita, Y.; Shirakusa, T. Opposite effects of thrombospondin-1 via CD36 and CD47 on homotypic aggregation of monocytic cells. Matrix Biol. 2002, 21, 441–448.

273. Bonnefoy, A.; Daenens, K.; Feys, H.B.; De Vos, R.; Vandervoort, P.; Vermylen, J.; Lawler, J.; Hoylaerts, M.F. Thrombospondin-1 controls vascular platelet recruitment and thrombus adherence in mice by protecting (sub)endothelial VWF from cleavage by ADAMTS13. Blood 2006, 107, 955–964.

274. Voit, S.; Udelhoven, M.; Lill, G.; Aktas, B.; Nieswandt, B.; Schror, K.; Weber, A.A. The C-terminal peptide of thrombospondin-1 stimulates distinct signaling pathways but induces an activation-independent agglutination of platelets and other cells. FEBS Lett. 2003, 544, 240–245.

275. Farrow, B.; Berger, D.H.; Rowley, D. Tumor-derived pancreatic stellate cells promote pancreatic cancer cell invasion through release of thrombospondin-2. J. Surg. Res. 2009, 156, 155–160.

276. Ghoneim, C.; Soula-Rothhut, M.; Rothhut, B. Thrombospondin-1 in differentiated thyroid cancer: Dr. Jekyll and Mr. Hyde. Connect. Tissue Res. 2008, 49, 257–260.

277. Sid, B.; Langlois, B.; Sartelet, H.; Bellon, G.; Dedieu, S.; Martiny, L. Thrombospondin-1 enhances human thyroid carcinoma cell invasion through urokinase activity. Int. J. Biochem. Cell Biol. 2008, 40, 1890–1900.
278. Albo, D.; Berger, D.H.; Rothman, V.L.; Tuszynski, G.P. Role of urokinase plasminogen activator receptor in thrombospondin 1-mediated tumor cell invasion. *J. Surg. Res.* **1999**, *82*, 331–338.

279. Robinet, A.; Emonard, H.; Banyai, L.; Laronze, J.Y.; Patthy, L.; Hornebeck, W.; Bellon, G. Collagen-binding domains of gelatinase A and thrombospondin-derived peptides impede endocytic clearance of active gelatinase A and promote HT1080 fibrosarcoma cell invasion. *Life Sci.* **2008**, *82*, 376–382.

280. Rath, G.M.; Schneider, C.; Dedieu, S.; Rothhut, B.; Soula-Rothhut, M.; Ghoneim, C.; Sid, B.; Morjani, H.; El Btaouri, H.; Martiny, L. The C-terminal CD47/IAP-binding domain of thrombospondin-1 prevents camptothecin- and doxorubicin-induced apoptosis in human thyroid carcinoma cells. *Biochim. Biophys. Acta* **2006**, *1763*, 1125–1134.

© 2010 by the authors; licensee MDPI, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).