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Thrombospondin-based Antiangiogenic Therapy

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Abstract

Thrombospondins (TSPs) are a family of extracellular matrix proteins that regulate tissue genesis and remodeling. TSP-1 plays a pivotal role in the regulation of both physiological and pathological angiogenesis. The inhibitory effects of TSP-1 on angiogenesis have been established in numerous experimental models. Among other TSP members, TSP-2 has equivalent domain structure as TSP-1 and shares most functions of TSP-1. The mechanisms by which TSP-1 and -2 inhibit angiogenesis can be broadly characterized as direct effects on vascular endothelial cells and indirect effects on the various angiogenic regulators. The fact that TSP-1 and -2 are potent endogenous angiogenic inhibitors has prompted studies to explore their therapeutic applications, and detailed understanding of the mechanisms of action of TSP-1 and -2 has facilitated the design of therapeutic strategies to optimize these activities. The therapeutic effects can be achieved by up-regulation of endogenous TSPs, or by the delivery of recombinant proteins or synthetic peptides that contain sequences from the angiogenic domain of TSP-1. In this article, we review the progress in thrombospondin-based anti-angiogenic therapy and discuss the perspectives on the significant challenges that remain.

Keywords

thrombospondin; angiogenesis; antiangiogenic therapy; endothelial cell

Introduction

Thrombospondins (TSPs) are a family of five multidomain, calcium-binding extracellular glycoproteins that are synthesized, secreted, and incorporated into the extracellular matrix of a wide variety of normal and transformed cells of both mesenchymal and epithelial origin (Adams and Lawler, 2004). Thrombospondin-1 (TSP-1) was the first identified and therefore the prototypic member of the family, and has been studied most intensively. TSP-1 is the first protein to be shown to play a critical role as a naturally occurring inhibitor of angiogenesis. Of the members of TSP family, TSP-2 has equivalent domain structure as TSP-1 and is also a potent inhibitor of angiogenesis.

The expression of TSP-1 in adult tissue is limited for the most part to sites of tissue remodeling. At these sites, TSP-1 acts in the pericellular space to regulate cellular phenotype and extracellular matrix structure. Virtually every domain of TSP-1 has a receptor on the cell

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surface. The specific repertoire of receptors that a given cell expresses may determine its response to TSP-1. Whereas TSP-1 promotes the migration of vascular smooth muscle cells, it is a potent inhibitor of endothelial cell migration. TSP-1 modulates extracellular matrix structure by binding to matrix proteins such as fibronectin and collagen, and by modulating the activity of extracellular proteinase such as matrix metalloproteinases (MMPs) and plasmin. The ability to regulate cellular phenotype and extracellular matrix structure enable TSP-1 and -2 to be key regulators of the tissue remodeling that is associated with angiogenesis, development, wound healing, and synaptogenesis. They are also involved in the pathological tissue remodeling that is associated with atherosclerosis, neoplasia and tumor angiogenesis.

The TSPs can be divided into structural domains that reflect exon shuffling during evolution (Figure 1) (Chen et al., 2000). TSP-1 and -2 have an N-terminal domain of approximately 200 amino acids that contains a high-affinity-binding site for heparin and heparan sulfate proteoglycans. This domain also mediates the uptake and clearance of the TSPs through a low-density lipoprotein receptor-related protein (LRP)-dependent mechanism. Three copies of the thrombospondin type 1 repeat (TSR) and three copies of the epidermal growth factor (EGF), or type 2, repeat are found in the middle of TSP-1 and -2. The TSRs are found in approximately 100 proteins in the human genome (Tucker, 2004). The TSRs have been shown to inhibit tumor angiogenesis and growth (Lawler and Detmar, 2004). Furthermore, a therapeutic agent, designated ABT-510, is based on an eight-amino-acid sequence within the second TSR (see below) (Haviv et al., 2005). The TSRs of TSP-1 bind to $\beta 1$ integrins, CD36 and transforming growth factor (TGF) β . Whereas TSP-1 and -2 probably have similar functions, they differ in their ability to activate TGF β . Activation of TGF β requires the sequence RFK between the first and second TSR of TSP-1 (Young and Murphy-Ullrich, 2004b). Since TSP-2 lacks this sequence, it is not able to activate TGF β . The C-terminal portion of TSP-1 and -2 (including the sequence from the last type 2 repeat to the C-terminal of the protein) is composed of a series of contiguous calcium binding sites that are wrapped around a β sandwich structure that is formed by the last 200 amino acids of the proteins (Carlson et al., 2005). This C-terminal domain is highly conserved in all five members of the thrombospondin gene family and thus has been designated the thrombospondin signature domain. This domain appears to bind 30 calcium ions, suggesting that the TSPs are involved in calcium homeostasis within the cell; however, this function of the thrombospondins has not been explored in detail.

Mechanisms of Inhibition of Angiogenesis by TSP-1 and -2

Numerous *in vitro* and *in vivo* approaches have been used to identify multiple mechanisms by which TSP-1 and -2 inhibit angiogenesis. These mechanisms can be broadly characterized as having direct effects on inhibiting endothelial cell migration and inducing endothelial cell apoptosis, or indirect effects on the various growth factors, cytokines and proteases that regulate angiogenesis. A detailed understanding of the mechanisms of action of TSP-1 and -2 has facilitated the design of therapeutic strategies to optimize these activities.

Direct Effects of TSP-1 on Endothelial Cell Function

TSP-1 and -2 are potent inhibitors of endothelial cell migration induced by vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF). This activity has been mapped to the TSRs (Lawler and Detmar, 2004). In an early study, a second sequence in the procollagen homology region was also reported to inhibit migration (Tolsma et al., 1993); however, the function of this sequence has not been characterized further. Three different synthetic peptides from the TSRs inhibit endothelial cell migration (Figure 1) (Iruela-Arispe et al., 1999). The sequence KRFKQDGGWSHWSPWSSC has been modified in various ways to increase its half-life in circulation (Guo et al., 1997). These peptide analogs inhibit angiogenesis in a rat model of retinopathy of prematurity and tumor growth in orthotopic models of breast and brain cancer (Bogdanov et al., 1999; Guo et al., 1997; Shafiee et al., 2000). It is interesting to note

that this sequence inhibits the proliferation of both the endothelial cells and the tumor cells through a TGF β -independent mechanism. The anti-angiogenic therapeutic agent ABT-510 is based on the sequence GVITRIR in the second β -strand of TSR2 (Reiher et al., 2002; Westphal, 2004). Together, the three sequences comprise a continuous region in the N-terminal half of the second TSR of TSP-1 (Figure 1). It should be noted that all three TSRs of TSP-1 and -2 have similar sequences and that peptides from the first and third TSRs of TSP-1 also have inhibitory effects on endothelial cells (Tolsma et al., 1993). Whereas TSP-2 has not been studied as extensively, the active sequences in TSP-1 are conserved in TSP-2. In the structure of TSRs, the active sequences are in two anti-parallel β -strands and the intervening turn (Tan et al., 2002). The arginines within the second β -strand orient their side chains so that they interdigitate with three tryptophans on the first β -strand to form cation- π bonds. In this way, the three active sequences are arranged in a positively charged surface patch on the second TSR. This surface patch is thought to represent the binding site for the various ligands of the TSRs, including TGF β and CD36.

TSRs reportedly inhibit endothelial cell migration through their interaction with CD36 (Simantov and Silverstein, 2003) and integrins (Short et al., 2005). CD36 is an 88,000-Da membrane protein that mediates the uptake of oxidized lipids and the anti-angiogenic activity of TSP-1 and -2 (Dawson et al., 1997). The interaction of CD36 with TSP-1 down modulates the VEGF receptor-2 and p38 MAP kinase phosphorylation that is induced by VEGF-A, and thus antagonizes the function of VEGF (Primo et al., 2005). CD36 is enriched in cholesterol-rich lipid rafts, where it associates with the src family kinases Fyn, Lyn and Yes (Simantov and Silverstein, 2003). As discussed below, Fyn is in the signaling pathway that leads to induction of endothelial cell apoptosis in response to TSP-1. CD36 also associates with integrins and the tetraspanin, CD9 (Miao et al., 2001b). The physiological significance of this association is not known; however, β 1 integrins have recently been reported to mediate the inhibition of large-vessel endothelial cell migration by the TSRs (Short et al., 2005). Because the large-vessel endothelial cells lack CD36 on the cell membrane, these data indicate that the interaction of TSRs and β 1 integrins inhibits the migration of endothelial cells in a CD36-independent manner. This effect is specific for β 1 integrins, because integrin β 3-siRNA has no effect on the inhibitory efficacy of TSRs. Antagonists of α 3 and α 5 integrin subunits and phosphoinositol 3 kinase (PI3K) block the inhibition of large vessel endothelial cell migration by the TSRs. Taken together, the data suggest that CD36 and β 1 integrins may collaborate to form a receptor complex for the TSRs that functions to inhibit endothelial cell migration. The data also raise the possibility that TSPs function by directing the assembly of multiprotein complexes on the cell surface.

The interaction of the TSRs with CD36 also results in the induction of endothelial cell apoptosis (Jimenez et al., 2000). Fyn and c-Jun N-terminal kinase (JNK) appear to be essential for this process, as TSP-1 is inactive in Fyn-null or JNK-1-null mice (Jimenez et al., 2000; Jimenez et al., 2001). This pathway also involves p38 MAPK and the up-regulation of Fas ligand (Volpert et al., 2002b). Quiescent endothelial cells express low levels of Fas receptor; however, its expression is increased in response to stimulation by VEGF. The increase in Fas ligand in response to TSP-1 leads to signals that counter the pro-survival signals of VEGF. These data suggest that TSP-1 potentiates the normal vessel regression that is associated with decreased VEGF expression. This conclusion is consistent with the observation that vessel density is increased in TSP-1-null mice during the catagen phase of the hair follicle cycle (Yano et al., 2003). Since tumor blood vessels are formed in response to VEGF and other growth factors and cytokines, the up-regulation of Fas ligand may target these new blood vessels in particular. TSP-1 has also been shown to attenuate the survival pathways of endothelial cells (Nor et al., 2000). TSP-1 mediates endothelial cell apoptosis and inhibits angiogenesis in association with increased expression of Bax and decreased expression of Bcl-2. TSP1 also down-regulates VEGF-mediated Bcl-2 expression in endothelial cells (Nor et al., 2000). Besides well-

documented in vitro data on TSP-1 induced microvascular endothelial cell apoptosis, TSP-1 has been shown to induce tumor vessel endothelial cell apoptosis in vivo (Zhang et al., 2005). A three-fold increase in tumor endothelial cell apoptosis is observed within pancreatic tumors when mice are systemically treated with the TSRs (Zhang et al., 2005). Whereas various proteins have been shown to be involved in TSP-1-induced apoptosis of endothelial cells, the details of the signaling pathway remain to be determined. A better understanding of this pathway would facilitate the design of optimal therapeutic approaches.

Both TSP-1 and TSP-2 inhibit the proliferation of human microvascular endothelial cells by induction of cell death and inhibition of cell cycle progression (Armstrong et al., 2002). TSP-1 and TSP-2 have a similar ability to block the cell cycle progression induced by multiple mitogens, including bFGF, EGF, IGF-1, and VEGF, and to arrest the cells in the G₀/G₁ phase. This inhibition of cell cycle progression appears to be independent of caspase activation or cell death induction. CD36 seems to be important to the cell cycle arrest effects of TSPs, because neither TSP-1 nor TSP-2 inhibits the growth of human umbilical vein endothelial cells, which lack CD36 expression.

TSP-1 also exerts its antiangiogenic effects by directly blocking nitric oxide (NO)-driven proangiogenic responses (Roberts et al., 2007). Multiple proangiogenic factors, including VEGF, angiopoietin-1, insulin, and estrogen, elicit endothelial NO synthase (eNOS) phosphorylation, which leads to sustained low fluxes of NO and consequently stimulates endothelial cell proliferation via activating cGMP pathway. Binding of TSP-1 to CD36 or CD47 is sufficient to inhibit NO-induced cGMP synthesis, and TSP-1 also inhibits the downstream angiogenesis effector pathways of cGMP. More importantly, in the presence of physiologic levels of NO, vascular cells become hypersensitive to the inhibitory effects of TSP-1, suggesting that low doses of NO donors may synergize with TSP-1-based antiangiogenic therapies.

Some portions of the endothelial cells that form the tumor microvasculature are reportedly derived from circulating endothelial progenitor cells (Rafii et al., 2002; Shaked et al., 2005). These cell populations are approximately two-fold higher in TSP-1-null mice, suggesting that endogenous levels of TSP-1 suppress production and/or survival of these circulating endothelial progenitor cells. In addition, ABT-510 decreases the levels of circulating endothelial cells in TSP-1-null mice suggesting that TSP-1- and TSR-based therapeutic agents may inhibit angiogenesis by decreasing the level of circulating endothelial cells. (Shaked et al., 2005).

Indirect Effects of TSP-1 on Endothelial Cell Function

Besides inhibiting VEGF receptor-2 phosphorylation through CD36, TSP-1 inhibits angiogenesis by antagonizing VEGF mobilization from the extracellular matrix and by binding directly to VEGF (Gupta et al., 1999; Rodriguez-Manzaneque et al., 2001). Inhibition of VEGF activity involves the TSRs and other domains of TSP-1. The N-terminal heparin-binding domain of TSP-1 reportedly competes with VEGF for binding sites on endothelial cell proteoglycans (Gupta et al., 1999; Margosio et al., 2003). TSP-1 also binds to FGF-2 and hepatocyte growth factor/scatter factor, suggesting that it functions as a scavenger for angiogenic growth factors (Margosio et al., 2003). The precise domain for growth factor binding has not been identified. The complex formed by TSP-1 and VEGF can be cleared from the pericellular space through an LRP-dependent mechanism (Greenaway et al., 2007).

Matrix-bound VEGF is released by the proteolytic activity of matrix metalloproteinases (MMPs). TSP-1 and -2 reportedly down-regulate the activity and increase the rate of clearance of MMPs (Bein and Simons, 2000; Yang et al., 2001). The level of active MMP-9 in mammary tumor tissue is inversely correlated to the amount of TSP-1 (Rodriguez-Manzaneque et al.,

2001). When TSP-1 is over-expressed, the amount of VEGF that is associated with VEGF receptor-2 is decreased and the vessel size and number is decreased within the mammary tumors. In gliomas, the concomitant binding of TSP-1, MMP-2 and LRP results in decreased angiogenesis (Fears et al., 2005). The original yeast-two-hybrid data indicates that the TSRs bind to the type II fibronectin repeats of MMP-2 (Bein and Simons, 2000). Thus, TSP-1 inhibits the activation of MMPs and facilitates their uptake and clearance.

TSP-1 also indirectly influences angiogenesis through the activation of TGF β . Whereas the precise mechanism underlying the activation of TGF β by TSP-1 is not fully understood, the amino acid sequence RFK between the first and second TSRs of TSP-1 is essential (Young and Murphy-Ullrich, 2004a). Since the other TSPs do not have this sequence, TSP-1 is the only member of the thrombospondin family that can activate TGF β . The effect of TGF β on angiogenesis is complex, involving both positive and negative effects (Bertolino et al., 2005). In A431 experimental tumors, over-expression of the TSRs results in an increase in active TGF β (Yee et al., 2004). Only constructs that contain the RFK sequence increase active TGF β and decrease vessel size, suggesting that the TGF β that is activated by TSP-1 can inhibit angiogenesis in this model.

Therapeutic Applications

Many pathologic conditions have been proven to be angiogenesis dependent and therefore amenable to antiangiogenic treatment (Folkman, 1995). The molecular mechanisms underlying the regulation of tumor angiogenesis, including the role of TSP-1 and -2, are perhaps the most intensively studied among all diseases. This section will focus on the therapeutic applications of TSP-1 and -2 in antiangiogenic treatment of cancer.

Tumor angiogenesis is regulated by a dynamic balance between angiogenic stimulators and inhibitors. The acquisition of an angiogenic phenotype by tumor cells involves up-regulation of pro-angiogenic factors and the down-regulation of endogenous inhibitors. Down-regulation of TSP-1 in tumor cells is a frequent step toward the acquisition of an angiogenic phenotype. In many tumors, down-regulation of TSP-1 accompanies activation of oncogenes or inactivation of tumor suppressor genes. Whereas oncogenes tend to suppress TSP-1 expression, tumor suppressor genes commonly increase TSP-1 synthesis. Thus, tumor tissue acts to promote angiogenesis by increasing stimulators of angiogenesis, like VEGF, and by decreasing endogenous inhibitors of angiogenesis. Ras, Myc, Id1, src, c-Jun and HER2 reportedly repress TSP-1 expression (Janz et al., 2000; Mettouchi et al., 1994; Rak et al., 2000; Slack and Bornstein, 1994; Volpert et al., 2002a; Watnick et al., 2003; Wen et al., 2006). Hyperactivation of oncogenic Ras leads to activation of PI3K, Rho, Rho-associated kinase (ROCK) and Myc, and decreased TSP-1 expression (Watnick et al., 2003). HER2 suppresses p38 MAP kinase and consequently decreased p38 kinase-activated TSP-1 expression (Wen et al., 2006). Down-regulation of TSP-1 by Myc appears to involve increased TSP-1 mRNA turnover more than suppression of transcription (Janz et al., 2000). The tumor cells also appear to secrete factors that result in decreased TSP-1 expression in neighboring stromal fibroblasts (Kalas et al., 2005). Ras-transformed fibrosarcoma cells secrete low-molecular-weight, heat-labile, trypsin-resistant factors that down-regulate TSP-1 in nontumorigenic dermal fibroblast cell lines (Kalas et al., 2005). More importantly, TSP-1 expression is down-regulated in tumor-derived endothelial cells by the PI3K/Akt/mTOR pathway, resulting in increased survival and angiogenic properties in these cells (Bussolati et al., 2006). In contrast, tumor suppressor genes like p53, PTEN and Smad4 act to increase TSP-1 expression (Dameron et al., 1994; Schwarte-Waldhoff et al., 2000; Su et al., 2003; Volpert et al., 1997). Loss of their function again leads to a shift in the balance to favor the pro-angiogenic factors. Many gene alterations that lead to decreased TSP-1 expression also lead to increased expression of VEGF, although possibly through distinct signaling pathways. For example, mutations in ras, HER2 or p53 affect both

sides of the angiogenic balance to facilitate the acquisition of a pro-angiogenic phenotype in tumors (Hanahan and Weinberg, 2000; Rak et al., 2000; Volpert et al., 1997; Watnick et al., 2003; Wen et al., 2006). In this way, the tumor cells create a microenvironment that is permissive for growth factor-induced angiogenesis. In addition, tumor hypoxia may also inhibit TSP-1 and induce VEGF expression and thus promote the switch to an angiogenic phenotype (Laderoute et al., 2000).

The fact that TSP-1 is a potent endogenous inhibitor of angiogenesis that is often down-regulated in tumor tissue has prompted several groups to explore therapeutic applications of TSP-1. These efforts fall into two basic approaches, the identification of strategies to up-regulate endogenous TSP-1, and the delivery of recombinant TSRs or synthetic peptides that contain sequences from the TSRs.

Strategies for Up-Regulation of TSP-1 or -2

The continuous administration of low doses of chemotherapeutics is sometimes referred to as metronomic dosing or anti-angiogenic chemotherapy (Browder et al., 2000). Metronomic dosing with cyclophosphamide increases the circulating levels of TSP-1 (Bocci et al., 2003). The source of the TSP-1 is controversial in that one group identifies endothelial cells as the source, and others propose that the tumor cells secrete elevated TSP-1 (Bocci et al., 2003; Damber et al., 2006; Hamano et al., 2004). This effect is specific to TSP-1 in that TSP-2 was not found to be up-regulated by low dose chemotherapy in a rat prostate cancer model (Damber et al., 2006). Metronomic dosing may also sensitize endothelial cells to TSP-1-induced apoptosis by up-regulating Fas receptor expression. Since TSP-1 and ABT-510 increase Fas ligand, metronomic chemotherapy increases the efficacy of ABT-510 (Yap et al., 2005). To show that the inhibition of tumor growth is due to increased TSP-1, the efficacy of metronomic dosing has been compared in wild-type and TSP-1-null mice. Whereas metronomic dosing with cyclophosphamide inhibits the growth of experimental Lewis lung carcinomas in wild-type mice, it is not effective in TSP-1-null mice (Bocci et al., 2003). Since metronomic dosing therapy depends on TSP-1 up-regulation, the circulating level of TSP-1 has been proposed as a marker for its efficacy. However, in a clinical trial with thirty-three children with recurrent, refractory solid tumors who received celecoxib and either low dose metronomic vinblastine or cyclophosphamide, the circulating levels of TSP-1, as well as many other angiogenic regulators, were highly variable and did not correlate with disease progression or maintenance of stable disease (Stempak et al., 2006). Thus, further clinical studies are necessary to integrate the data from animal models with those from humans.

The implantation of a biodegradable polymer that contains TSP-2 over-expressing fibroblasts into the ovarian pedicle produces elevated systemic levels of TSP-2 for at least 5 weeks (Streit et al., 2002). In these experiments, the increased TSP-2 inhibited the growth of squamous cell carcinomas, melanomas and Lewis lung carcinomas. The decreased tumor growth was associated with a decrease in tumor vessel size and an increase in tumor cell apoptosis. Systemic delivery of a recombinant protein containing the N-terminal domain through the TSRs of TSP-2 also inhibits the growth and angiogenesis of experimental squamous cell carcinomas (Noh et al., 2003).

TSR-based Therapeutic Approaches

Systemic injection of purified platelet TSP-1 inhibits B16F10 experimental tumor growth (Miao et al., 2001a). Because TSP-1 is a large molecule with multiple functional domains, several groups have sought to define a region of the molecule that is anti-angiogenic and more amenable to mass production and therapeutic use. Synthetic peptides that include sequences from the procollagen homology region and from the TSRs inhibit angiogenesis (Dawson and Bouck, 1999; Guo et al., 1997; Iruela-Arispe et al., 1999; Reiher et al., 2002; Tolsma et al.,

1993). In addition, systemic injections of a recombinant version of all three TSRs of TSP-1, designated 3TSR, inhibits experimental pancreatic tumors, melanoma and Lewis lung carcinoma (Miao et al., 2001a; Zhang et al., 2005). Inhibition of pancreatic tumor growth is associated with decreased intratumoral vessel number and size, as well as increased endothelial cell apoptosis (Zhang et al., 2005). To identify essential amino acids for the inhibition of tumor growth by TSP-1, we have prepared a recombinant version of the second TSR of TSP-1 with (designated TSR2+RFK) and without (designated TSR2) the RFK sequence that is essential for the activation of TGF β (Miao et al., 2001a). TSR2 is a potent inhibitor of angiogenesis and growth of experimental and Lewis lung carcinoma tumors. Inclusion of the RFK sequence results in the activation of TGF β and a further inhibition of B16F10 melanoma, but not Lewis lung carcinoma. This difference results from the fact that the B16F10 melanoma cells are responsive to the growth inhibitory effects of TGF β , while the Lewis lung carcinomas are not (Miao et al., 2001a).

Evidence suggests that a consistent level of angiogenic inhibitors might improve the therapeutic potency and efficacy of cancer treatment (Capillo et al., 2003; Drixler et al., 2000; Kisker et al., 2001; Morishita et al., 1995; Zhang et al., 2006). Continuous delivery of 3TSR through mini-osmotic pumps is more effective than daily bolus injections (Zhang et al., 2006), indicating the therapeutic benefit of sustained levels of angiogenic inhibitors. These data provide the rationale for gene therapy strategies using vectors which are capable of sustained, long-term transgene expression. Adeno-associated virus-mediated angiogenic gene therapy with 3TSR has been developed in our laboratory showing significant antiangiogenic and anti-tumor efficacy (unpublished data).

Two TSR mimetic peptides, ABT-510 and ABT-526, have been developed for antiangiogenic treatment. ABT-510 is based on the sequence GVITRIR in the second β -strand of TSR2 (Reiher et al., 2002; Westphal, 2004). This sequence has been modified in several ways to improve its stability in circulation. The N- and C-terminals have been capped, the isoleucine is replaced by D-allo-isoleucine and the first arginine is replaced by norvaline. The second arginine is essential for the activity of ABT-510 (Reiher et al., 2002). ABT-526 is equivalent to ABT-510 except that the D-allo-isoleucine is replaced by D-isoleucine. ABT-510 is more soluble in water and has a longer half-life in primate circulation than ABT-526 (Westphal, 2004). *In vivo* studies show that both peptides slow tumor growth in syngeneic and xenograft mouse models (Haviv et al., 2005; Reiher et al., 2002). In a prospective trial using ABT-510 or ABT-526 in pet dogs with naturally occurring cancers, no significant treatment-related adverse effects were observed in any dog. Anti-tumor activity was similar for both peptides. Nine of 58 dogs with various types of tumors that were treated with ABT-526 were found to have stable disease, seven displayed partial response, and complete response was observed in three dogs. Among 122 dogs treated with ABT-510, there were three complete response, six partial response, and fourteen with stable disease (Rusk et al., 2006).

The safety, pharmacokinetics and pharmacodynamic results of a phase I clinical trial of ABT-510 have been reported (Hoekstra et al., 2005). ABT-510 was administered subcutaneously as a bolus injection either once or twice daily with dose escalation beginning with 100 mg/24 h. Patients in a continuous-infusion arm of the study developed erythema and edema of the skin at the infusion site which was sometimes painful and resulted in this arm of the study being discontinued. Of the 35 patients who received bolus injections of ABT-510, three adverse events were considered potentially related to the drug treatment: a fatal intracranial hemorrhage, a transient ischemic attack and a case of new-onset diabetes mellitus. However, a causal link between ABT-510 treatment and the adverse event could not be unequivocally made in all three cases. ABT-510 has a half-life of 1.1 ± 0.2 h in circulation (Hoekstra et al., 2005). These data suggest that multiple treatment during a fixed period of time or continuous administration will be more effective than increasing the amount of ABT-510

in a single dose. Whereas the phase I clinical trial is not designed to determine efficacy, some patients appeared to benefit from ABT-510 treatment. Prolonged stable disease was observed in patients with sarcoma, renal cell carcinoma, carcinoma of the cervix, colorectal carcinoma or a germ cell tumor. Phase II clinical trials are currently underway with ABT-510 as a single agent or in combination with chemotherapy for the treatment of soft tissue sarcoma, renal cell carcinoma, lymphoma and non-small-cell lung cancer.

CD36 expression on endothelial cells is increased by peroxisome proliferating-activated receptor (PPAR γ) ligands, such as troglitazone. Since CD36 is reportedly the receptor for ABT-510, the effect of a combination of troglitazone and ABT-510 on experimental bladder carcinomas has been investigated (Huang et al., 2004). Troglitazone increased CD36 expression in tumor-associated capillaries. Whereas ABT-510 or troglitazone alone inhibit tumor growth by about 30%, the combination of these reagents inhibited tumor growth by 74%. As anticipated, the mean vessel density and the degree of endothelial cell apoptosis are significantly greater with the combination therapy than with ABT-510 alone.

Taken together, the data indicate that ABT-510 has the potential to be an effective therapeutic agent with low toxicity. However, it should be noted that the preclinical and clinical trials have not involved treatment for long periods of time. Besides microvascular endothelial cells, the receptor for ABT-510, CD36, is also expressed on platelets, monocytes, hematopoietic cells and breast epithelial cells. It is important to assess the effect of long-term ABT-510 treatment on these cell types. This is especially true for treatment strategies that sensitize the tumor microvasculature to ABT-510 by up-regulating CD36. In addition, the effect of ABT-510 on physiological angiogenesis, for example during wound healing, needs to be fully evaluated.

Combination of TSP-1 based agents with other anti-cancer treatments

Combination therapy using TSP-1 or ABT-510 with other treatment modalities or other anti-angiogenic reagents has been shown to be effective in various murine models of cancer. Endostatin and TSP-1 act through different receptors and affect the expression of different genes, suggesting that they have distinct modes of action (Cline et al., 2002). Thus, a combination of endostatin and a TSR-based peptide that is a predecessor of ABT-510 effectively inhibits angiogenesis and experimental Lewis lung carcinoma growth (Cline et al., 2002).

Treatment with TSP-1 has been shown to improve the efficacy of radiation therapy (Rofstad et al., 2003). When TSP-1 is injected before radiation treatment, it increases endothelial cell apoptosis in the tumor microvasculature and reduces the fraction of radiobiologically hypoxic cells (Rofstad et al., 2003). Moreover, TSP-1 treatment has been proven to be useful after surgical tumor removal or curative radiation treatment. In D-12 melanoma models, surgical resection as well as curative radiation treatment of the primary tumor resulted in accelerated growth of dormant micrometastases, because the primary tumors suppress the growth of pulmonary micrometastases by secreting TSP-1 into the blood circulation. This growth could be prevented by treating the host with exogenous TSP-1 after surgery or irradiation (Rofstad et al., 2004), indicating that cancer patients with TSP-1-producing primary tumors may benefit from combined local treatment and antiangiogenic/antimetastatic treatment with TSP-1 based agents to prevent both the growth of dormant metastases and recurrence of primary tumors.

Low doses of cyclophosphamide, cisplatin or docetaxel also reportedly increase endothelial cell Fas receptor (Yap et al., 2005). TSP-1 and ABT-510 increase the level of Fas ligand on endothelial cells (Volpert et al., 2002b; Yap et al., 2005). Thus, the combination of chemotherapy agents and TSP-1 or ABT-510 enhanced the induction of endothelial cell apoptosis and the inhibition of tumor angiogenesis.

The combinations of ABT-510 with gemcitabine-cisplatin or 5-fluorouracil-leucovorin have been studied in two phase I clinical trials (Gietema et al., 2006; Hoekstra et al., 2006). No clinically significant pharmacokinetic interactions between ABT-510 and the chemotherapeutic agents were observed, and adding ABT-510 does not appear to increase toxicity.

Besides its antiangiogenic function, TSP-1 has also been shown to sensitize prostate cancer cells to the cytotoxicity of taxane (Lih et al., 2006). A recently identified gene, *txr1*, impedes taxane-induced apoptosis in tumor cells by transcriptionally down-regulating the production of TSP-1, whereas treatment with TSP-1 or a TSP-1 mimetic peptide sensitized cells to taxane cytotoxicity by activating signaling through the interaction of the C-terminus of TSP-1 with CD47 (Lih et al., 2006). These data provide a new direction for combinatory treatment with TSP-1 and chemotherapy. Since chemoresistance is a common phenomenon in many types of cancer, whether TSP-1 will work as a chemosensitizer to other chemotherapeutic agents or in other cancers needs to be further studies.

Future Considerations for the Development of TSP-1-based Therapies

The data obtained to date indicate that TSP-1 is a potent inhibitor of tumor angiogenesis with considerable therapeutic potential. TSP-1 levels can be increased by direct delivery of the protein or by stimulating its expression in tissue. The anti-angiogenic activity of TSP-1 can be mimicked by recombinant proteins or peptides that contain TSR sequences. These reagents are considerably easier and less expensive to produce than the intact TSP-1 molecule. Since multiple TSR sequences seem to be important, and because the tertiary structure of the TSRs may be necessary for optimal orientation of these sequences, the recombinant proteins may be more active than the synthetic peptides. Whereas a sequence in the procollagen homology region has been shown to inhibit angiogenesis, the role of this domain in CD36 binding has not been explored. Since the procollagen homology region is adjacent to the TSRs, it is possible that both domains contribute to the anti-angiogenic activity of TSP-1.

Whereas the structure of the TSRs has been elucidated, very little is known about the structure of CD36. An understanding of the structure of CD36, and the nature of its interaction with the TSRs, will facilitate the identification of small-molecule ligands of CD36 that can mimic the effect of TSP-1 binding. In addition, a more complete understanding of the signaling pathways that are affected by CD36 and the identification of points of crosstalk between the CD36 signaling pathway and that of VEGF will identify novel targets. It has been indicated that $\beta 1$ integrins act as a receptor system involved in the inhibition of endothelial cell migration by the TSRs (Short et al., 2005), and this observation identifies two areas of further investigation that will be important for the development of TSR-based therapeutics. The relative importance of CD36 and $\beta 1$ integrins for inhibition of angiogenesis by TSP-1 needs to be determined. Furthermore, since $\beta 1$ integrins are widely expressed, it will be important to carefully search for side effects of long-term TSR treatment.

Drug resistance is a common occurrence in chemotherapy because the genome of the cancer cell is unstable. Since the endothelial cell is the target of anti-angiogenesis therapy, it is possible that drug resistance would not limit this approach; however, tumor angiogenesis results from a dialog between the cancer cell and the endothelium. To stimulate angiogenesis, tumor cells express VEGF, and other pro-angiogenic factors, and down-regulate TSP-1. The decrease in TSP-1 also serves to relieve the growth-inhibitory effect of TGF β . Fibrosarcomas have been shown to overcome the inhibitory effects of TSP-1 by increasing VEGF expression and by becoming resistant to TGF β (Filleur et al., 2001). Although over-expression of TSP-1 in the fibrosarcoma cells initially inhibits tumor growth, eventually the tumors do grow. Cells from these tumors grow rapidly when they are harvested and re-injected into rats. Thus, in the

presence of TSP-1, it appears that a population of tumor cells that were resistant to its inhibitory effects was selected. Significant inhibition of tumor growth can be achieved by concomitant up-regulation of TSP-1 and down-regulation of VEGF (Filleur et al., 2003).

Whereas the role of TSP-1 as an inhibitor of primary tumor growth is well documented, its role in tumor metastasis is controversial. TSP-1 expression correlates with a non-metastatic phenotype in two clones that were derived from the MDA-MB-435 breast cancer cell line (Urquidi et al., 2002). The non-metastatic cell line NM-2C5 displays a 15-fold increase in TSP-1 expression compared to the metastatic cell line M-4A4. In another study, transfection of full-length TSP-1 into MDA-MB-435 cells decreased the growth of orthotopic experimental tumors and decreased the number of pulmonary metastases by approximately 50% (Weinstat-Saslow et al., 1994). However, in vitro studies suggest TSP-1 promotes the invasion of breast cancer cell lines through collagen gels (Wang et al., 1996). TSP-1 has also been shown to increase pancreatic cancer cell migration and invasion in vitro through up-regulating the plasminogen/plasmin system (Albo et al., 1998). If TSP-1 is indeed prometastatic, it will be important to develop strategies for the inhibition of angiogenesis and primary tumor growth that do not promote metastatic spread.

Reference

- Adams JC, Lawler J. The thrombospondins. *Int J Biochem Cell Biol* 2004;36:961–8. [PubMed: 15094109]
- Albo D, Berger DH, Tuszynski GP. The effect of thrombospondin-1 and TGF-beta 1 on pancreatic cancer cell invasion. *J Surg Res* 1998;76:86–90. [PubMed: 9695745]
- Armstrong LC, Bjorkblom B, Hankenson KD, Siadak AW, Stiles CE, Bornstein P. Thrombospondin 2 inhibits microvascular endothelial cell proliferation by a caspase-independent mechanism. *Mol Biol Cell* 2002;13:1893–905. [PubMed: 12058057]
- Bein K, Simons M. Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. *J Biol Chem* 2000;275:32167–73. [PubMed: 10900205]
- Bertolino P, Deckers M, Lebrin F, ten Dijke P. Transforming growth factor-beta signal transduction in angiogenesis and vascular disorders. *Chest* 2005;128:585S–590S. [PubMed: 16373850]
- Bocci G, Francia G, Man S, Lawler J, Kerbel RS. Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. *Proc Natl Acad Sci U S A* 2003;100:12917–22. [PubMed: 14561896]
- Bogdanov A Jr, Marecos E, Cheng HC, Chandrasekaran L, Krutzsch HC, Roberts DD, Weissleder R. Treatment of experimental brain tumors with thrombospondin-1 derived peptides: an in vivo imaging study. *Neoplasia* 1999;1:438–45. [PubMed: 10933059]
- Browder T, Butterfield CE, Kraling BM, Shi B, Marshall B, O'Reilly MS, Folkman J. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res* 2000;60:1878–86. [PubMed: 10766175]
- Bussolati B, Assenzio B, Deregibus MC, Camussi G. The proangiogenic phenotype of human tumor-derived endothelial cells depends on thrombospondin-1 downregulation via phosphatidylinositol 3-kinase/Akt pathway. *J Mol Med* 2006;84:852–63. [PubMed: 16924472]
- Capillo M, Mancuso P, Gobbi A, Monestiroli S, Pruneri G, Dell'Agnola C, Martinelli G, Shultz L, Bertolini F. Continuous infusion of endostatin inhibits differentiation, mobilization, and clonogenic potential of endothelial cell progenitors. *Clin Cancer Res* 2003;9:377–82. [PubMed: 12538491]
- Carlson CB, Bernstein DA, Annis DS, Misenheimer TM, Hannah BL, Mosher DF, Keck JL. Structure of the calcium-rich signature domain of human thrombospondin-2. *Nat Struct Mol Biol* 2005;12:910–4. [PubMed: 16186819]
- Chen H, Herndon ME, Lawler J. The cell biology of thrombospondin-1. *Matrix Biol* 2000;19:597–614. [PubMed: 11102749]
- Cline EI, Biccato S, DiBello C, Lingen MW. Prediction of in vivo synergistic activity of antiangiogenic compounds by gene expression profiling. *Cancer Res* 2002;62:7143–8. [PubMed: 12499246]

- Damber JE, 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;58:354–60. [PubMed: 16333676]
- Dameron KM, Volpert OV, Tainsky MA, Bouck N. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* 1994;265:1582–4. [PubMed: 7521539]
- Dawson, DW.; Bouck, NP. Thrombospondin as an inhibitor of angiogenesis. In: Teicher, BA., editor. *Antiangiogenic agents in cancer therapy*. Humana; Totowa, NJ: 1999. p. 185-203.
- Dawson DW, Pearce SF, Zhong R, Silverstein RL, Frazier WA, Bouck NP. CD36 mediates the in vitro inhibitory effects of thrombospondin-1 on endothelial cells. *J Cell Biol* 1997;138:707–17. [PubMed: 9245797]
- Drixler TA, Borel Rinkes IH, Ritchie ED, van Vroonhoven TJ, Gebbink MF, Voest EE. Continuous administration of angiostatin inhibits accelerated growth of colorectal liver metastases after partial hepatectomy. *Cancer Res* 2000;60:1761–5. [PubMed: 10749151]
- Fears CY, Grammer JR, Stewart JE Jr, Annis DS, Mosher DF, Bornstein P, Gladson CL. Low-density lipoprotein receptor-related protein contributes to the antiangiogenic activity of thrombospondin-2 in a murine glioma model. *Cancer Res* 2005;65:9338–46. [PubMed: 16230396]
- Filleul S, Courtin A, Ait-Si-Ali S, Guglielmi J, Merle C, Harel-Bellan A, Clezardin P, Cabon F. SiRNA-mediated inhibition of vascular endothelial growth factor severely limits tumor resistance to antiangiogenic thrombospondin-1 and slows tumor vascularization and growth. *Cancer Res* 2003;63:3919–22. [PubMed: 12873985]
- Filleul S, Volpert OV, Degeorges A, Voland C, Reiher F, Clezardin P, Bouck N, Cabon F. In vivo mechanisms by which tumors producing thrombospondin 1 bypass its inhibitory effects. *Genes Dev* 2001;15:1373–82. [PubMed: 11390357]
- Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995;1:27–31. [PubMed: 7584949]
- Gietema JA, Hoekstra R, de Vos FY, Uges DR, van der Gaast A, Groen HJ, Loos WJ, Knight RA, Carr RA, Humerickhouse RA, Eskens FA. 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;17:1320–7. [PubMed: 16728485]
- 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;210:807–18. [PubMed: 17154366]
- Guo NH, Krutzsch HC, Inman JK, Shannon CS, Roberts DD. Antiproliferative and antitumor activities of D-reverse peptides derived from the second type-1 repeat of thrombospondin-1. *J Pept Res* 1997;50:210–21. [PubMed: 9309585]
- Gupta K, Gupta P, Wild R, Ramakrishnan S, Hebbel RP. Binding and displacement of vascular endothelial growth factor (VEGF) by thrombospondin: effect on human microvascular endothelial cell proliferation and angiogenesis. *Angiogenesis* 1999;3:147–58. [PubMed: 14517432]
- Hamano Y, Sugimoto H, Soubasakos MA, Kieran M, Olsen BR, 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;64:1570–4. [PubMed: 14996710]
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
- Haviv F, Bradley MF, Kalvin DM, Schneider AJ, Davidson DJ, Majest SM, McKay LM, Haskell CJ, Bell RL, Nguyen B, Marsh KC, Surber BW, Uchic JT, Ferrero J, Wang YC, Leal J, Record RD, Hodde J, Badylak SF, Lesniewski RR, 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;48:2838–46. [PubMed: 15828822]
- Hoekstra R, de Vos FY, Eskens FA, de Vries EG, Uges DR, Knight R, Carr RA, Humerickhouse R, Verweij J, Gietema JA. Phase I study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 with 5-fluorouracil and leucovorin: a safe combination. *Eur J Cancer* 2006;42:467–72. [PubMed: 16406507]
- Hoekstra R, de Vos FY, Eskens FA, Gietema JA, van der Gaast A, Groen HJ, Knight RA, Carr RA, Humerickhouse RA, Verweij J, de Vries EG. 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;23:5188–97. [PubMed: 16051960]
- Huang H, Campbell SC, Bedford DF, Nelius T, Veliceasa D, Shroff EH, Henkin J, Schneider A, Bouck N, Volpert OV. Peroxisome proliferator-activated receptor gamma ligands improve the antitumor efficacy of thrombospondin peptide ABT510. *Mol Cancer Res* 2004;2:541–50. [PubMed: 15498928]
- Iruela-Arispe ML, Lombardo M, Kruttsch HC, Lawler J, Roberts DD. Inhibition of angiogenesis by thrombospondin-1 is mediated by 2 independent regions within the type 1 repeats. *Circulation* 1999;100:1423–31. [PubMed: 10500044]
- Janz A, Seignani C, Kenyon K, Ngo CV, Thomas-Tikhonenko A. Activation of the myc oncoprotein leads to increased turnover of thrombospondin-1 mRNA. *Nucleic Acids Res* 2000;28:2268–75. [PubMed: 10871348]
- Jimenez B, Volpert OV, Crawford SE, Febbraio M, Silverstein RL, Bouck N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nat Med* 2000;6:41–8. [PubMed: 10613822]
- Jimenez B, Volpert OV, 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;20:3443–8. [PubMed: 11423995]
- Kalas W, Yu JL, Milsom C, Rosenfeld J, Benezra R, Bornstein P, Rak J. Oncogenes and Angiogenesis: down-regulation of thrombospondin-1 in normal fibroblasts exposed to factors from cancer cells harboring mutant ras. *Cancer Res* 2005;65:8878–86. [PubMed: 16204059]
- Kisker O, Becker CM, Prox D, Fannon M, D'Amato R, Flynn E, Fogler WE, Sim BK, Allred EN, Pirie-Shepherd SR, Folkman J. Continuous administration of endostatin by intraperitoneally implanted osmotic pump improves the efficacy and potency of therapy in a mouse xenograft tumor model. *Cancer Res* 2001;61:7669–74. [PubMed: 11606410]
- Laderoute KR, Alarcon RM, Brody MD, Calaoagan JM, Chen EY, Knapp AM, Yun Z, Denko NC, Giaccia AJ. Opposing effects of hypoxia on expression of the angiogenic inhibitor thrombospondin 1 and the angiogenic inducer vascular endothelial growth factor. *Clin Cancer Res* 2000;6:2941–50. [PubMed: 10914744]
- Lawler J, Detmar M. Tumor progression: the effects of thrombospondin-1 and -2. *Int J Biochem Cell Biol* 2004;36:1038–45. [PubMed: 15094119]
- Lih CJ, Wei W, Cohen SN. Tsr1: a transcriptional regulator of thrombospondin-1 that modulates cellular sensitivity to taxanes. *Genes Dev* 2006;20:2082–95. [PubMed: 16847352]
- 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;102:4399–406. [PubMed: 12947001]
- Mettouchi A, Cabon F, Montreau N, Vernier P, Mercier G, Blangy D, Tricoire H, Vigier P, Binetruy B. SPARC and thrombospondin genes are repressed by the c-jun oncogene in rat embryo fibroblasts. *Embo J* 1994;13:5668–78. [PubMed: 7988564]
- Miao WM, Seng WL, 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* 2001a;61:7830–9. [PubMed: 11691800]
- Miao WM, Vasile E, Lane WS, Lawler J. CD36 associates with CD9 and integrins on human blood platelets. *Blood* 2001b;97:1689–96. [PubMed: 11238109]
- Morishita T, Mii Y, Miyauchi Y, Miura S, Honoki K, Aoki M, Kido A, Tamai S, Tsutsumi M, Konishi Y. Efficacy of the angiogenesis inhibitor O-(chloroacetyl-carbamoyl)fumagillol (AGM-1470) on osteosarcoma growth and lung metastasis in rats. *Jpn J Clin Oncol* 1995;25:25–31. [PubMed: 7745819]
- Noh YH, Matsuda K, Hong YK, Kunstfeld R, Riccardi L, Koch M, Oura H, Dadras SS, Streit M, Detmar M. An N-terminal 80 kDa recombinant fragment of human thrombospondin-2 inhibits vascular endothelial growth factor induced endothelial cell migration in vitro and tumor growth and angiogenesis in vivo. *J Invest Dermatol* 2003;121:1536–43. [PubMed: 14675207]
- Nor JE, Mitra RS, Sutorik MM, Mooney DJ, Castle VP, Polverini PJ. Thrombospondin-1 induces endothelial cell apoptosis and inhibits angiogenesis by activating the caspase death pathway. *J Vasc Res* 2000;37:209–18. [PubMed: 10859480]

- 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;19:1713–5. [PubMed: 16037098]
- Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nat Rev Cancer* 2002;2:826–35. [PubMed: 12415253]
- Rak J, Mitsuhashi Y, Sheehan C, Tamir A, Vilorio-Petit A, Filmus J, Mansour SJ, Ahn NG, Kerbel RS. Oncogenes and tumor angiogenesis: differential modes of vascular endothelial growth factor up-regulation in ras-transformed epithelial cells and fibroblasts. *Cancer Res* 2000;60:490–8. [PubMed: 10667605]
- Reiher FK, Volpert OV, Jimenez B, Crawford SE, Dinney CP, Henkin J, Haviv F, Bouck NP, Campbell SC. Inhibition of tumor growth by systemic treatment with thrombospondin-1 peptide mimetics. *Int J Cancer* 2002;98:682–9. [PubMed: 11920636]
- Roberts DD, Isenberg JS, Ridnour LA, Wink DA. Nitric oxide and its gatekeeper thrombospondin-1 in tumor angiogenesis. *Clin Cancer Res* 2007;13:795–8. [PubMed: 17289869]
- Rodriguez-Manzanique JC, Lane TF, Ortega MA, Hynes RO, Lawler J, Iruela-Arispe ML. Thrombospondin-1 suppresses spontaneous tumor growth and inhibits activation of matrix metalloproteinase-9 and mobilization of vascular endothelial growth factor. *Proc Natl Acad Sci U S A* 2001;98:12485–90. [PubMed: 11606713]
- Rofstad EK, Galappathi K, Mathiesen B. Thrombospondin-1 treatment prevents growth of dormant lung micrometastases after surgical resection and curative radiation therapy of the primary tumor in human melanoma xenografts. *Int J Radiat Oncol Biol Phys* 2004;58:493–9. [PubMed: 14751520]
- Rofstad EK, Henriksen K, Galappathi K, Mathiesen B. Antiangiogenic treatment with thrombospondin-1 enhances primary tumor radiation response and prevents growth of dormant pulmonary micrometastases after curative radiation therapy in human melanoma xenografts. *Cancer Res* 2003;63:4055–61. [PubMed: 12874006]
- Rusk A, McKeegan E, Haviv F, Majest S, Henkin J, Khanna C. Preclinical evaluation of antiangiogenic thrombospondin-1 peptide mimetics, ABT-526 and ABT-510, in companion dogs with naturally occurring cancers. *Clin Cancer Res* 2006;12:7444–55. [PubMed: 17189418]
- Schwarte-Waldhoff I, Volpert OV, Bouck NP, Sipos B, Hahn SA, Klein-Scory S, Luttges J, Kloppel G, Graeven U, Eilert-Micus C, Hintelmann A, Schmiegeler W. Smad4/DPC4-mediated tumor suppression through suppression of angiogenesis. *Proc Natl Acad Sci U S A* 2000;97:9624–9. [PubMed: 10944227]
- Shafiee A, Penn JS, Kruttsch HC, Inman JK, Roberts DD, Blake DA. Inhibition of retinal angiogenesis by peptides derived from thrombospondin-1. *Invest Ophthalmol Vis Sci* 2000;41:2378–88. [PubMed: 10892887]
- Shaked Y, Bertolini F, Man S, Rogers MS, Cervi D, Foutz T, Rawn K, Voskas D, Dumont DJ, Ben-David Y, Lawler J, Henkin J, Huber J, Hicklin DJ, D'Amato RJ, Kerbel RS. Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; Implications for cellular surrogate marker analysis of antiangiogenesis. *Cancer Cell* 2005;7:101–11. [PubMed: 15652753]
- Short SM, Derrien A, Narsimhan RP, Lawler J, Ingber DE, Zetter BR. Inhibition of endothelial cell migration by thrombospondin-1 type-1 repeats is mediated by beta1 integrins. *J Cell Biol* 2005;168:643–53. [PubMed: 15716381]
- Simantov R, Silverstein RL. CD36: a critical anti-angiogenic receptor. *Front Biosci* 2003;8:s874–82. [PubMed: 12957861]
- Slack JL, Bornstein P. Transformation by v-src causes transient induction followed by repression of mouse thrombospondin-1. *Cell Growth Differ* 1994;5:1373–80. [PubMed: 7696186]
- Stempak D, Gammon J, Halton J, Moghrabi A, Koren G, Baruchel S. A pilot pharmacokinetic and antiangiogenic biomarker study of celecoxib and low-dose metronomic vinblastine or cyclophosphamide in pediatric recurrent solid tumors. *J Pediatr Hematol Oncol* 2006;28:720–8. [PubMed: 17114958]
- Streit M, Stephen AE, Hawighorst T, Matsuda K, Lange-Asschenfeldt B, Brown LF, Vacanti JP, Detmar M. Systemic inhibition of tumor growth and angiogenesis by thrombospondin-2 using cell-based antiangiogenic gene therapy. *Cancer Res* 2002;62:2004–12. [PubMed: 11929817]

- Su JD, Mayo LD, Donner DB, Durden DL. PTEN and phosphatidylinositol 3'-kinase inhibitors up-regulate p53 and block tumor-induced angiogenesis: evidence for an effect on the tumor and endothelial compartment. *Cancer Res* 2003;63:3585–92. [PubMed: 12839945]
- Tan K, Duquette M, Liu JH, Dong Y, Zhang R, Joachimiak A, Lawler J, Wang JH. Crystal structure of the TSP-1 type 1 repeats: a novel layered fold and its biological implication. *J Cell Biol* 2002;159:373–82. [PubMed: 12391027]
- Tolsma SS, Volpert OV, Good DJ, Frazier WA, Polverini PJ, 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. [PubMed: 7686555]
- Tucker RP. The thrombospondin type 1 repeat superfamily. *Int J Biochem Cell Biol* 2004;36:969–74. [PubMed: 15094110]
- Urquidí V, Sloan D, Kawai K, Agarwal D, Woodman AC, Tarin D, Goodison S. Contrasting expression of thrombospondin-1 and osteopontin correlates with absence or presence of metastatic phenotype in an isogenic model of spontaneous human breast cancer metastasis. *Clin Cancer Res* 2002;8:61–74. [PubMed: 11801541]
- Volpert OV, Dameron KM, Bouck N. Sequential development of an angiogenic phenotype by human fibroblasts progressing to tumorigenicity. *Oncogene* 1997;14:1495–502. [PubMed: 9136993]
- Volpert OV, Pili R, Sikder HA, Nelius T, Zaichuk T, Morris C, Shiflett CB, Devlin MK, Conant K, Alani RM. Id1 regulates angiogenesis through transcriptional repression of thrombospondin-1. *Cancer Cell* 2002a;2:473–83. [PubMed: 12498716]
- Volpert OV, Zaichuk T, Zhou W, Reiher F, Ferguson TA, Stuart PM, Amin M, Bouck NP. Inducer-stimulated Fas targets activated endothelium for destruction by anti-angiogenic thrombospondin-1 and pigment epithelium-derived factor. *Nat Med* 2002b;8:349–57. [PubMed: 11927940]
- Wang TN, Qian X, Granick MS, Solomon MP, Rothman VL, Berger DH, Tuszynski GP. Thrombospondin-1 (TSP-1) promotes the invasive properties of human breast cancer. *J Surg Res* 1996;63:39–43. [PubMed: 8661169]
- Watnick RS, Cheng YN, Rangarajan A, Ince TA, Weinberg RA. Ras modulates Myc activity to repress thrombospondin-1 expression and increase tumor angiogenesis. *Cancer Cell* 2003;3:219–31. [PubMed: 12676581]
- Weinstat-Saslow DL, Zabrenetzky VS, VanHoutte K, Frazier WA, Roberts DD, Steeg PS. Transfection of thrombospondin 1 complementary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. *Cancer Res* 1994;54:6504–11. [PubMed: 7527299]
- Wen XF, Yang G, Mao W, Thornton A, Liu J, Bast RC Jr, Le XF. HER2 signaling modulates the equilibrium between pro- and antiangiogenic factors via distinct pathways: implications for HER2-targeted antibody therapy. *Oncogene* 2006;25:6986–96. [PubMed: 16715132]
- Westphal JR. Technology evaluation: ABT-510, Abbott. *Curr Opin Mol Ther* 2004;6:451–7. [PubMed: 15468604]
- Yang Z, Strickland DK, Bornstein P. Extracellular matrix metalloproteinase 2 levels are regulated by the low density lipoprotein-related scavenger receptor and thrombospondin 2. *J Biol Chem* 2001;276:8403–8. [PubMed: 11113133]
- Yano K, Brown LF, Lawler J, Miyakawa T, Detmar M. Thrombospondin-1 plays a critical role in the induction of hair follicle involution and vascular regression during the catagen phase. *J Invest Dermatol* 2003;120:14–9. [PubMed: 12535193]
- Yap R, Veliceasa D, Emmenegger U, Kerbel RS, McKay LM, Henkin J, Volpert OV. Metronomic low-dose chemotherapy boosts CD95-dependent antiangiogenic effect of the thrombospondin peptide ABT-510: a complementation antiangiogenic strategy. *Clin Cancer Res* 2005;11:6678–85. [PubMed: 16166447]
- Yee KO, 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;165:541–52. [PubMed: 15277228]
- Young GD, Murphy-Ullrich JE. Molecular interactions that confer latency to transforming growth factor-beta. *J Biol Chem* 2004a;279:38032–9. [PubMed: 15208302]

- Young GD, Murphy-Ullrich JE. The tryptophan-rich motifs of the thrombospondin type 1 repeats bind VLAL motifs in the latent transforming growth factor-beta complex. *J Biol Chem* 2004b;279:47633–42. [PubMed: 15342643]
- Zhang X, Caitlin 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.* 2006In press
- 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;11:2337–44. [PubMed: 15788685]

Figure 1.

The structure of TSP-1 and the TSRs. TSP-1 comprises multiple functional domains and each domain has different receptors. The amino acid sequence of the second type 1 repeat (TSR + RFK) is shown, and the active amino acid sequences and the anti-angiogenic peptides are indicated.