Thrombospondin-1-Based Antiangiogenic Therapy

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Abstract

Ocular angiogenesis is one of the underlying causes of blindness and vision impairment and may occur in a spectrum of disorders, including diabetic retinopathy, neovascular age-related macular degeneration, retinal artery or vein occlusion, and retinopathy of prematurity. As such, strategies to inhibit angiogenesis by suppressing vascular endothelial growth factor activity have proven to be effective in the clinic for the treatment of eye diseases. A complementary approach would be to increase the level of naturally occurring inhibitors of angiogenesis, such as thrombospondin (TSP)-1. This article summarizes the development of TSP-1-based inhibitors of angiogenesis.

ANGIOGENESIS IS A tightly regulated process that involves the formation of new blood vessels from preexisting vasculature. This process requires migration, proliferation, and survival of endothelial cells, as well as vessel maturation, vessel remodeling, and degradation of the extracellular matrix (ECM).¹ Under normal conditions, the endothelial cell number is stable without significant proliferation, mostly due to the relative balance between proangiogenic factors such as vascular endothelial growth factor (VEGF) and antiangiogenic factors^{2,3} such as thrombospondin (TSP)-1 and TSP-2.⁴ When there is a preponderance of proangiogenic factors, pathogenic consequences arise; moreover, these consequences may lead to the occurrence and progression of several human disorders, such as cancer, inflammation, atherosclerosis, and blinding eye diseases.^{5,6}

Angiogenesis affects almost all tissues of the eye, including the cornea, iris, retina, and choroid.^{7,8} Ocular angiogenesis is one of the underlying causes of blindness and vision impairment and may occur in a spectrum of ocular disorders, including diabetic retinopathy, neovascular age-related macular degeneration (AMD), retinal artery or vein occlusion, and retinopathy of prematurity.^{9,10} Delicate and leaky vasculature is formed during imbalanced ocular angiogenesis. This increased vascular permeability induces retinal edema, fibrovascular proliferation with tractional and rhegmatogenous retinal detachment or vascular fragility resulting in hemorrhage, and an accumulation of fluids and protein exudates in ocular cavities, minimizing the transparency of the cornea and impairing the structure and function of retinal neurons.^{3,10,11}

The main therapeutic approaches currently available for ocular neovascular diseases attempt to inhibit new vessel formation and leaky vasculature. VEGF is an angiogenic

factor and vital mediator of physiological and pathological neovascularization; moreover, it is critical for the proliferation and migration of endothelial cells as well as for the accurate vascular patterning and tube formation during retinal vascular development.^{3,12–14} Also, an imbalance in the VEGF-related pathway is correlated with corneal neovascularization.^{13,15} Therefore, several antiVEGF drugs, such as pegaptanib (Macugen®; Valeant), bevacizumab (Avastin®; Genentech), ranibizumab (Lucentis®; Genentech), and aflibercept (Eylea®; Regeneron) are currently being used for ocular neovascular diseases.¹⁶ All these antiangiogenic drugs target the ligand–receptor interaction of the VEGF signaling pathway.³ Pegaptanib is a pegylated aptamer that binds to the 165 isoform of VEGF, which is the principle isoform responsible for pathological ocular angiogenesis.¹⁷ Bevacizumab is a recombinant humanized monoclonal antibody that binds to extracellular VEGF preventing interaction with VEGF receptors (VEGFRs).¹⁸ Ranibizumab is a recombinant humanized monoclonal antibody fragment that neutralizes all active forms of VEGF-A.¹⁹ Aflibercept is a VEGF Trap and is a fusion protein that binds to all isoforms of the VEGF-A family.²⁰

Anti-VEGF agents have revolutionized the treatment of pathological neovascularization diseases. Even though clinical trials have shown the efficacy of these agents in visual improvements for ocular neovascular disorders, serious adverse side effects can occur due to the repeated and long-term use that is normally needed.^{16,21–23} VEGF is essential for the survival of quiescent endothelial cells and glial cells.³ Therefore, systemic complications can arise from suppressing VEGF signaling. Major systemic side effects that have been observed are hypertension, myocardial infarction, stroke, delayed wound healing, and nonocular

hemorrhage.¹⁶ Ocular complications, including intraocular inflammation, infectious endophthalmitis, hemorrhage, and retinal detachment, have occurred from intravitreal injections of anti-VEGF agents.^{3,16} Therefore, it is imperative to develop therapeutic approaches that do not fully suppress VEGF signaling in the eye, but perhaps allows for the proper maintenance of sufficient VEGF.

TSPs are a family of 5 genes (TSP-1 to TSP-5) encoding large glycoproteins that bind to specific receptors expressed on a wide variety of cells and regulate multiple ECM functions. TSPs modulate several tissue-specific processes by interacting with growth factors, cytokines, cell surface receptors, and components of ECM. There are 2 subgroups of the TSP protein family subgroups A and B.²⁴ TSP-1 and TSP-2 comprise subgroup A and possess thrombospondin type 1 repeats (TSRs).²⁴ Subgroup B (TSP-3 to TSP-5) family members do not have TSRs.²⁵ The TSRs interact with CD36, a fatty acid translocase on the endothelial cell membrane to mediate angiogenesis.^{26–28} TSP-1 is the only member of the TSP family that contains the KRFK amino acid sequence required for transforming growth factor- β (TGF- β) activation.²⁹ TGF- β is essential for ocular immune privilege, which is diminished in TSP-1-deficient mice.³⁰ Moreover, TSP-1 is critical for the antigen-presenting cells that aid in eye immune privilege by producing peripheral tolerance.³¹

The expression of TSP-1 can be observed in several ocular cell types and compartments, including the cornea,³² conjunctiva,³³ iris and ciliary body,³⁴ retina,³⁵ choroidal vascular membrane,³⁶ retinal pigment epithelial cells,³⁶ and glial cells.³⁷ In addition, TSP-1 is detectable in the aqueous^{38,39} and vitreous humor.³⁹ TSP-1 plays a significant role in ocular functions, including the maintenance of corneal avascularity.³⁴ Moreover, TSP-1 mediates several cellular processes, such as angiogenesis, cell migration, wound healing, activation, and regulation of TGF- β during inflammatory immune responses. TSP-1 null mice show abnormal lacrimal glands and eye surface defects similar to human Sjogren's syndrome.⁴⁰ Furthermore, irreversible damage of the retina is seen in experimental autoimmune uveitis in TSP-1-deficient mice.³⁰ In patients with diabetic retinopathy, TSP-1 is routinely low in the vitreous fluid; thus, TSP-1 plays a role in the alterations in the angiogenic balance of the eye.⁴¹ Attenuation of proliferation and migration of retinal pericytes is seen in TSP-1-deficient mice. In addition, AMD patients express decreased levels of TSP-1 in Bruch's membrane and choriocapillaris, which may result in a permissive environment for choroidal neovascularization (CNV).⁴² These observations reveal that TSP-1 plays a significant role in ocular homeostasis and function and has the potential to be used as a therapeutic agent to fulfill unmet clinical needs in neovascular ocular disease.

Since TSP-1 is a large protein, considerable effort has been made to identify small active sites that would be more amenable to drug development than the intact protein. Multiple domains within the protein have been found to modulate angiogenesis. The amino terminal domain reportedly promotes endothelial migration.⁴³ By contrast, TSRs have a potent inhibitory activity in a wide range of *in vitro* and *in vivo* angiogenesis assays using multiple different inducers, including fibroblast growth factor-2, interleukin-8, platelet-derived growth factor, and VEGF.⁴⁴ It is worth noting that the critical sequence for activation of TGF- β lies between the first and second TSRs of TSP-1.⁴⁵ In an early study, the anti-

angiogenic activity was also detected in a peptide from the procollagen homology domain; however, this observation has not been pursued further.⁴⁶ Another region of TSP-1, designated the type 3 repeats, inhibits angiogenesis by directly binding and sequestering fibroblast growth factor-2.⁴⁷ TSP-1, through its C-terminal domain, binds CD47; this leads to various events that alter angiogenesis, which include the disruption of constitutive association between CD47 and VEGFR2⁴⁸ as well as the inhibition of VEGF-dependent NO signaling.⁴⁹

There are 3 TSRs in TSP-1 and TSP-2.⁵⁰ In addition, the TSR motif is present in ~90 other proteins in the human genome, some of which have been shown to have the antiangiogenic activity. The structure represents a unique protein fold that is stabilized by 3 disulfide bonds, the stacking of tryptophan and arginine residues, and glycosylation.^{51,52} Peptide studies have identified a number of key amino acid sequences within the TSRs of TSP-1, which have the antiangiogenic activity.^{46,53} A 7 amino acid sequence was used as the basis for the drugs ABT-510, ABT-898, and CVX-045.^{54–56} ABT-510 was taken through phase 2 clinical trials for the treatment of various types of cancer. Whereas this drug was well-tolerated, it displayed insufficient clinical activity to justify further development.^{57–59} The unique 3-dimensional fold of the TSRs and the weak activity of ABT-510 suggest that it is difficult to mimic the full antiangiogenic activity of the TSRs with small peptides and that recombinant versions of the TSRs may be a more effective alternative.

A recombinant version of all 3 TSRs of TSP-1, designated 3TSR, has been shown to be a potent inhibitor of angiogenesis and tumor growth in preclinical models of pancreatic, skin, colon, breast, brain, and ovarian cancer.^{60–64} In addition, 3TSR inhibits VEGF-induced vascular permeability.⁶⁵ The protein is soluble and stable *in vivo*. It can be delivered by intravenous or intraperitoneal injection, adeno-associated virus, or cell-based strategies.^{61,63,64} The latter 2 approaches eliminate the need to prepare injectable protein. The inhibition of pancreatic tumor growth by 3TSR is comparable to that of gemcitabine, a current first-line therapy.⁶⁶ In most cases, the inhibition of tumor growth by 3TSR is due to inhibition of angiogenesis, with no direct effects on the tumor cells. However, 3TSR reportedly inhibits the growth of brain and ovarian cancer cells through direct interaction with CD36, which is expressed on the tumor cells.^{63,64} The ability of 3TSR to inhibit the survival of both ovarian cancer and endothelial cells results in more effective inhibition of tumor growth and an increase in survival over that seen with chemotherapy in a preclinical model.⁶³ Further increases in survival are seen when 3TSR is combined with chemotherapy. These data indicate that specific antiangiogenic therapies may be uniquely suited for specific types of cancer, depending on the role of various signal transduction pathways. It is worth noting that 3TSR is significantly more active than ABT-898 or ABT-510 in preclinical models of ovarian cancer.^{55,63,67}

Whereas the vast majority of studies with 3TSR have focused on the treatment of cancer, these data indicate that 3TSR could be an effective inhibitor of angiogenesis in the eye. Indeed, ABT-898 is a potent inhibitor of tumor growth in experimental models of uveal melanoma.⁶⁸ Furthermore, intravitreal injection of ABT-898 inhibits CNV induced by laser injury by ~43% compared with vehicle-treated

mice.⁶⁹ These data indicate that preclinical studies should be undertaken to determine whether or not 3TSR could be used to treat AMD and diabetic retinopathy, either as a single agent or in conjunction with anti-VEGF therapy. Whereas anti-VEGF strategies have been remarkably successful for the treatment of neovascular AMD, some patients display a limited or no response to anti-VEGF therapy. Since 3TSR has a different mechanism of action, it may be effective in treating patients who do not respond to anti-VEGF therapy. As indicated above, TSP-1 appears to play a counter-regulatory function to VEGF during physiological angiogenesis. The concomitant use of 3TSR with anti-VEGF therapeutics would suppress pro-survival pathways and induce apoptotic pathways in endothelial cells and would thus target both sides of the angiogenic balance.

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Author Disclosure Statement

No competing financial interests exist.

References

- Qazi, Y., Maddula, S., and Ambati, B.K. Mediators of ocular angiogenesis. *J. Genet.* 88:495–515, 2009.
- Folkman, J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.* 1:27–31, 1995.
- Sulaiman, R.S., Basavarajappa, H.D., and Corson, T.W. Natural product inhibitors of ocular angiogenesis. *Exp. Eye Res.* 129C:161–171, 2014.
- Masli, S., Sheibani, N., Cursiefen, C., and Zieske, J. Matricellular protein thrombospondins: influence on ocular angiogenesis, wound healing and immunoregulation. *Curr. Eye Res.* 39:759–774, 2014.
- Carmeliet, P. Angiogenesis in life, disease and medicine. *Nature.* 438:932–936, 2005.
- Ellenberg, D., Azar, D.T., Hallak, J.A., Tobaigy, F., Han, K.Y., Jain, S., Zhou, Z., and Chang, J.H. Novel aspects of corneal angiogenic and lymphangiogenic privilege. *Prog. Retin. Eye Res.* 29:208–248, 2010.
- Markowska, A.I., Cao, Z., and Panjwani, N. Glycobiology of ocular angiogenesis. *Glycobiology.* 24:1275–1282, 2014.
- Adamis, A.P., Aiello, L.P., and D'Amato, R.A. Angiogenesis and ophthalmic disease. *Angiogenesis.* 3:9–14, 1999.
- Ferguson, T.A., and Apte, R.S. Angiogenesis in eye disease: immunity gained or immunity lost? *Semin. Immunopathol.* 30:111–119, 2008.
- Rajappa, M., Saxena, P., and Kaur, J. Ocular angiogenesis: mechanisms and recent advances in therapy. *Adv. Clin. Chem.* 50:103–121, 2010.
- Zhang, S.X., and Ma, J.X. Ocular neovascularization: implication of endogenous angiogenic inhibitors and potential therapy. *Prog. Retin. Eye Res.* 26:1–37, 2007.
- Dorrell, M.I., and Friedlander, M. Mechanisms of endothelial cell guidance and vascular patterning in the developing mouse retina. *Prog. Retin. Eye Res.* 25:277–295, 2006.
- Sene, A., Chin-Yee, D., and Apte, R.S. Seeing through VEGF: innate and adaptive immunity in pathological angiogenesis in the eye. *Trends Mol. Med.* 21:43–51, 2015.
- Dorrell, M.I., Aguilar, E., and Friedlander, M. Retinal vascular development is mediated by endothelial filopodia, a preexisting astrocytic template and specific R-cadherin adhesion. *Invest. Ophthalmol. Vis. Sci.* 43:3500–3510, 2002.
- Bock, F., Maruyama, K., Regenfuss, B., Hos, D., Steven, P., Heindl, L.M., and Cursiefen, C. Novel anti(lymph)angiogenic treatment strategies for corneal and ocular surface diseases. *Prog. Retin. Eye Res.* 34:89–124, 2013.
- Falavarjani, K.G., and Nguyen, Q.D. Adverse events and complications associated with intravitreal injection of anti-VEGF agents: a review of literature. *Eye (Lond.)* 27:787–794, 2013.
- Ng, E.W., Shima, D.T., Calias, P., Cunningham Jr., E.T., Guyer, D.R., and Adamis, A.P. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat. Rev. Drug Discov.* 5:123–132, 2006.
- Gunther, J.B., and Altaweel, M.M. Bevacizumab (Avastin) for the treatment of ocular disease. *Surv. Ophthalmol.* 54:372–400, 2009.
- Rosenfeld, P.J., Brown, D.M., Heier, J.S., Boyer, D.S., Kaiser, P.K., Chung, C.Y., Kim, R.Y.; MARINA Study Group. Ranibizumab for neovascular age-related macular degeneration. *N. Engl. J. Med.* 355:1419–1431, 2006.
- Stewart, M.W. Aflibercept (VEGF Trap-eye): the newest anti-VEGF drug. *Br. J. Ophthalmol.* 96:1157–1158, 2012.
- Ip, M.S., Scott, I.U., Brown, G.C., Brown, M.M., Ho, A.C., Huang, S.S., Recchia, F.M.; American Academy of Ophthalmology. Anti-vascular endothelial growth factor pharmacotherapy for age-related macular degeneration: a report by the American Academy of Ophthalmology. *Ophthalmology.* 115:1837–1846, 2008.
- Campochiaro, P.A., Heier, J.S., Feiner, L., Gray, S., Saroj, N., Rundle, A.C., Murahashi, W.Y., Rubio, R.G.; BRAVO Investigators. Ranibizumab for macular edema following branch retinal vein occlusion: six-month primary end point results of a phase III study. *Ophthalmology.* 117:1102–1112.e1, 2010.
- Nguyen, Q.D., Brown, D.M., Marcus, D.M., Boyer, D.S., Patel, S., Feiner, L., Gibson, A., Sy, J., Rundle, A.C., Hopkins, J.J., Rubio, R.G., Ehrlich, J.S.; RISE and RIDE Research Group. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. *Ophthalmology.* 119:789–801, 2012.
- Kazerounian, S., Yee, K.O., and Lawler, J. Thrombospondins in cancer. *Cell. Mol. Life Sci.* 65:700–712, 2008.
- Adams, J., and Lawler, J. Extracellular matrix: the thrombospondin family. *Curr. Biol.* 3:188–190, 1993.
- Ridnour, L.A., Thomas, D.D., Donzelli, S., Espey, M.G., Roberts, D.D., Wink, D.A., and Isenberg, J.S. The biphasic nature of nitric oxide responses in tumor biology. *Antioxid. Redox Signal.* 8:1329–1337, 2006.
- Jimenez, B., Volpert, O.V., Crawford, S.E., Febbraio, M., Silverstein, R.L., and Bouck, N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nat. Med.* 6:41–48, 2000.
- Dawson, D.W., Pearce, S.F., Zhong, R., Silverstein, R.L., Frazier, W.A., and Bouck, N.P. CD36 mediates the in vitro inhibitory effects of thrombospondin-1 on endothelial cells. *J. Cell Biol.* 138:707–717, 1997.
- Murphy-Ullrich, J.E., and Poczatek, M. Activation of latent TGF-beta by thrombospondin-1: mechanisms and physiology. *Cytokine Growth Factor Rev.* 11:59–69, 2000.

30. Zamiri, P., Masli, S., Kitaichi, N., Taylor, A.W., and Streilein, J.W. Thrombospondin plays a vital role in the immune privilege of the eye. *Invest. Ophthalmol. Vis. Sci.* 46:908–919, 2005.
31. Masli, S., Turpie, B., and Streilein, J.W. Thrombospondin orchestrates the tolerance-promoting properties of TGF β -treated antigen-presenting cells. *Int. Immunol.* 18:689–699, 2006.
32. Hiscott, P., Seitz, B., Schlotzer-Schrehardt, U., and Naumann, G.O. Immunolocalisation of thrombospondin 1 in human, bovine and rabbit cornea. *Cell Tissue Res.* 289: 307–310, 1997.
33. Gandhi, N.B., Su, Z., Zhang, X., Volpe, E.A., Pelegrino, F.S., Rahman, S.A., Li, D.Q., Pflugfelder, S.C., and de Paiva, C.S. Dendritic cell-derived thrombospondin-1 is critical for the generation of the ocular surface Th17 response to desiccating stress. *J. Leukoc. Biol.* 94:1293–1301, 2013.
34. Cursiefen, C., Masli, S., Ng, T.F., Dana, M.R., Bornstein, P., Lawler, J., and Streilein, J.W. Roles of thrombospondin-1 and -2 in regulating corneal and iris angiogenesis. *Invest. Ophthalmol. Vis. Sci.* 45:1117–1124, 2004.
35. Suzuma, K., Takagi, H., Otani, A., Oh, H., and Honda, Y. Expression of thrombospondin-1 in ischemia-induced retinal neovascularization. *Am. J. Pathol.* 154:343–354, 1999.
36. He, S., Incardona, F., Jin, M., Ryan, S.J., and Hinton, D.R. Thrombospondin-1 expression in RPE and choroidal neovascular membranes. *Yan Ke Xue Bao.* 22:265–274, 2006.
37. Cinatl Jr., J., Bittoova, M., Margraf, S., Vogel, J.U., Cinatl, J., Preiser, W., and Doerr, H.W. Cytomegalovirus infection decreases expression of thrombospondin-1 and -2 in cultured human retinal glial cells: effects of antiviral agents. *J. Infect. Dis.* 182:643–651, 2000.
38. Flugel-Koch, C., Ohlmann, A., Fuchshofer, R., Welge-Lüssen, U., and Tamm, E.R. Thrombospondin-1 in the trabecular meshwork: localization in normal and glaucomatous eyes, and induction by TGF- β 1 and dexamethasone in vitro. *Exp. Eye Res.* 79:649–663, 2004.
39. Sheibani, N., Sorenson, C.M., Cornelius, L.A., and Frazier, W.A. Thrombospondin-1, a natural inhibitor of angiogenesis, is present in vitreous and aqueous humor and is modulated by hyperglycemia. *Biochem. Biophys. Res. Commun.* 267:257–261, 2000.
40. Turpie, B., Yoshimura, T., Gulati, A., Rios, J.D., Dartt, D.A., and Masli, S. Sjogren's syndrome-like ocular surface disease in thrombospondin-1 deficient mice. *Am. J. Pathol.* 175:1136–1147, 2009.
41. Wang, S., Gottlieb, J.L., Sorenson, C.M., and Sheibani, N. Modulation of thrombospondin 1 and pigment epithelium-derived factor levels in vitreous fluid of patients with diabetes. *Arch. Ophthalmol.* 127:507–513, 2009.
42. Uno, K., Bhutto, I.A., McLeod, D.S., Merges, C., and Luty, G.A. Impaired expression of thrombospondin-1 in eyes with age related macular degeneration. *Br. J. Ophthalmol.* 90:48–54, 2006.
43. Dias, J.V., Benslimane-Ahmim, Z., Egot, M., Lokajczyk, A., Grelac, F., Galy-Fauroux, I., Juliano, L., Le-Bonniec, B., Takiya, C.M., Fischer, A.M., Blanc-Brude, O., Morandi, V., and Boisson-Vidal, C. A motif within the N-terminal domain of TSP-1 specifically promotes the proangiogenic activity of endothelial colony-forming cells. *Biochem. Pharmacol.* 84:1014–1023, 2012.
44. Lawler, P.R., and Lawler, J. Molecular basis for the regulation of angiogenesis by thrombospondin-1 and -2. *Cold Spring Harb. Perspect. Med.* 2:a006627, 2012.
45. Schultz-Cherry, S., Chen, H., Mosher, D.F., Misenheimer, T.M., Krutzsch, H.C., Roberts, D.D., and Murphy-Ullrich, J.E. Regulation of transforming growth factor- β activation by discrete sequences of thrombospondin 1. *J. Biol. Chem.* 270:7304–7310, 1995.
46. Tolsma, S.S., Volpert, O.V., Good, D.J., Frazier, W.A., Polverini, P.J., and Bouck, N. Peptides derived from two separate domains of the matrix protein thrombospondin-1 have anti-angiogenic activity. *J. Cell Biol.* 122:497–511, 1993.
47. Margosio, B., Rusnati, M., Bonezzi, K., Cordes, B.L., Annis, D.S., Urbinati, C., Giavazzi, R., Presta, M., Ribatti, D., Mosher, D.F., and Tarabozzi, G. Fibroblast growth factor-2 binding to the thrombospondin-1 type III repeats, a novel antiangiogenic domain. *Int. J. Biochem. Cell Biol.* 40:700–709, 2008.
48. Kaur, S., Martin-Manso, G., Pendrak, M.L., Garfield, S.H., Isenberg, J.S., and Roberts, D.D. Thrombospondin-1 inhibits VEGF receptor-2 signaling by disrupting its association with CD47. *J. Biol. Chem.* 285:38923–38932, 2010.
49. Miller, T.W., Isenberg, J.S., and Roberts, D.D. Thrombospondin-1 is an inhibitor of pharmacological activation of soluble guanylate cyclase. *Br. J. Pharmacol.* 159:1542–1547, 2010.
50. Adams, J.C., and Lawler, J. The thrombospondins. *Cold Spring Harb. Perspect. Biol.* 3:a009712, 2011.
51. Tan, K., Duquette, M., Liu, J.H., Dong, Y., Zhang, R., Joachimiak, A., Lawler, J., and Wang, J.H. Crystal structure of the TSP-1 type 1 repeats: a novel layered fold and its biological implication. *J. Cell Biol.* 159:373–382, 2002.
52. Vasudevan, D., Takeuchi, H., Johar, S.S., Majerus, E., and Haltiwanger, R.S. Peters plus syndrome mutations disrupt a noncanonical ER quality-control mechanism. *Curr. Biol.* 25:286–295, 2015.
53. Iruela-Arispe, M.L., Lombardo, M., Krutzsch, H.C., Lawler, J., and Roberts, D.D. Inhibition of angiogenesis by thrombospondin-1 is mediated by 2 independent regions within the type 1 repeats. *Circulation.* 100:1423–1431, 1999.
54. 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., Badyrak, S.F., Lesniewski, R.R., and Henkin, J. Thrombospondin-1 mimetic peptide inhibitors of angiogenesis and tumor growth: design, synthesis, and optimization of pharmacokinetics and biological activities. *J. Med. Chem.* 48:2838–2846, 2005.
55. Campbell, N., Greenaway, J., Henkin, J., and Petrik, J. ABT-898 induces tumor regression and prolongs survival in a mouse model of epithelial ovarian cancer. *Mol. Cancer Ther.* 10:1876–1885, 2011.
56. Li, L., Leedom, T.A., Do, J., Huang, H., Lai, J., Johnson, K., Osothpraprop, T.F., Rizzo, J.D., Doppalapudi, V.R., Bradshaw, C.W., Lappe, R.W., Woodnutt, G., Levin, N.J., and Pirie-Shepherd, S.R. Antitumor efficacy of a thrombospondin 1 mimetic CovX-body. *Transl. Oncol.* 4:249–257, 2011.
57. Ebbinghaus, S., Hussain, M., Tannir, N., Gordon, M., Desai, A.A., Knight, R.A., Humerickhouse, R.A., Qian, J., Gordon, G.B., and Figlin, R. Phase 2 study of ABT-510 in patients with previously untreated advanced renal cell carcinoma. *Clin. Cancer Res.* 13:6689–6695, 2007.
58. Gordon, M.S., Mendelson, D., Carr, R., Knight, R.A., Humerickhouse, R.A., Iannone, M., and Stopeck, A.T. A

- phase 1 trial of 2 dose schedules of ABT-510, an antiangiogenic, thrombospondin-1-mimetic peptide, in patients with advanced cancer. *Cancer*. 113:3420–3429, 2008.
59. Baker, L.H., Rowinsky, E.K., Mendelson, D., Humerickhouse, R.A., Knight, R.A., Qian, J., Carr, R.A., Gordon, G.B., and 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.* 26:5583–5588, 2008.
 60. Miao, W.M., Seng, W.L., Duquette, M., Lawler, P., Laus, C., and Lawler, J. Thrombospondin-1 type 1 repeat recombinant proteins inhibit tumor growth through transforming growth factor-beta-dependent and -independent mechanisms. *Cancer Res.* 61:7830–7839, 2001.
 61. Zhang, X., Xu, J., Lawler, J., Terwilliger, E., and Parangi, S. Adeno-associated virus-mediated antiangiogenic gene therapy with thrombospondin-1 type 1 repeats and endostatin. *Clin. Cancer Res.* 13:3968–3976, 2007.
 62. Yee, K.O., Connolly, C.M., Duquette, M., Kazerounian, S., Washington, R., and Lawler, J. The effect of thrombospondin-1 on breast cancer metastasis. *Breast Cancer Res. Treat.* 114:85–96, 2009.
 63. Russell, S., Duquette, M., Liu, J., Drapkin, R., Lawler, J., and Petrik, J. Combined therapy with thrombospondin-1 type I repeats (3TSR) and chemotherapy induces regression and significantly improves survival in a preclinical model of advanced stage epithelial ovarian cancer. *FASEB J.* 29:576–588, 2015.
 64. Choi, S.H., Tamura, K., Khajuria, R.K., Bhere, D., Neshterenko, I., Lawler, J., and Shah, K. Antiangiogenic variant of TSP-1 targets tumor cells in glioblastomas. *Mol. Ther.* 23:235–243, 2015.
 65. Zhang, X., Kazerounian, S., Duquette, M., Perruzzi, C., Nagy, J.A., Dvorak, H.F., Parangi, S., and Lawler, J. Thrombospondin-1 modulates vascular endothelial growth factor activity at the receptor level. *FASEB J.* 23:3368–3376, 2009.
 66. Zhang, X., Galardi, E., Duquette, M., Lawler, J., and Parangi, S. Antiangiogenic treatment with three thrombospondin-1 type 1 repeats versus gemcitabine in an orthotopic human pancreatic cancer model. *Clin. Cancer Res.* 11:5622–5630, 2005.
 67. Greenaway, J., Henkin, J., Lawler, J., Moorehead, R., and 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.* 8: 64–74, 2009.
 68. Wang, S., Neekhra, A., Albert, D.M., Sorenson, C.M., and Sheibani, N. Suppression of thrombospondin-1 expression during uveal melanoma progression and its potential therapeutic utility. *Arch. Ophthalmol.* 130:336–341, 2012.
 69. Wang, S., Sorenson, C.M., and Sheibani, N. Lack of thrombospondin 1 and exacerbation of choroidal neovascularization. *Arch. Ophthalmol.* 130:615–620, 2012.

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