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Review

Thrombospondin-1 in drug activity and tumor response to therapies

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ABSTRACT

Thrombospondins (TSPs) have numerous different roles in cancer, regulating the behavior of cancer cells and non-neoplastic cells, and defining the responses of tumor cells to environmental changes, thorough their ability to orchestrate cellular and molecular interactions in the tumor microenvironment (TME). As a result of these activities, TSPs can also control drug delivery and activity, tumor response and resistance to therapies, with different outcomes depending on the nature of TSP-interacting cell types, receptors, and ligands, in a highly context-dependent manner. This review, focusing primarily on TSP-1, discusses the effects of TSPs on tumor response to chemotherapy, antiangiogenic, low-dose metronomic chemotherapy, immunotherapy, and radiotherapy, by analyzing TSP activity on different cell compartments – tumor cells, vascular endothelial cells and immune cells. We review evidence of the value of TSPs, specifically TSP-1 and TSP-2, as biomarkers of prognosis and tumor response to therapy. Finally, we examine possible approaches to develop TSP-based compounds as therapeutic tools to potentiate the efficacy of anticancer therapy.

1. Introduction

The composition, mechanical properties and biological activities of the TME have a profound impact on different aspects of cancer progression, including response to therapy and drug activity. Factors influencing tumor cell-TME interactions, and particularly thrombospondins (TSPs) [1,2], can therefore affect not only the tumor's malignant behavior, but also the overall response of cancers to different therapeutic approaches.

In mammals, TSPs are a five-member family of matricellular glycoprotein, expressed by embryonic and adult cells following precise spatiotemporal regulation and are typically secreted and associated with the extracellular matrix. The homotrimeric TSP-1 and TSP-2, forming subgroup A, have similar structural and functional properties. Structurally, they are composed of a globular N-terminal domain, an oligomerization domain, a von Willebrand Factor type C/ pro-collagen domain, three type 1 repeats (properdin/thrombospondin repeats), three type 2 repeats (EGF-like), the type 3 repeats, calcium-binding domain and the C-terminal globular region. The pentameric TSP-3, TSP-4 and TSP-5/COMP, belonging to subgroup B, lack the pro-collagen and type 1 repeat domains and have a less developed N-terminal region. The modular structure enables TSPs to interact with a large variety of ligands, including growth factors, cell receptors, extracellular matrix components and proteases, making them pleiotropic. Depending on the local cellular and molecular environment, the activity of TSPs is highly context-dependent, determined by the presence of different ligands, cell receptors, soluble factors and proteases that ultimately control domain availability and activity [3].

TSPs function by interacting with multiple cell receptors, recognized by different TSP domains. TSP-1, the most studied member of the TSP family, interacts with the ubiquitous receptor CD47 (also known as integrin-associated protein), the fatty acid transporter CD36, several β1 and β3 integrins, the calreticulin/low-density lipoprotein receptor related protein 1 (LRP1) complex, and the neuronal receptor α2δ-1.

TSPs take part in a range of physiological and pathological processes including cancer. TSP-1 controls different phases of tumor development and malignant progression by directly modulating cancer cell behavior but also acting on the structural and functional properties of the TME. TSP-1-mediated modulation of the recruitment and activity of tumor-infiltrating cells such as endothelial cells, fibroblasts, and immune cells ultimately promotes or inhibits tumor progression, angiogenesis, matrix organization and immune response.

As a result of these activities on tumor cells and the TME, TSPs play a critical role in tumor response to therapies with different, even opposite

Abbreviations: TSP, thrombospondin; TME, tumor microenvironment; LDM, low-dose metronomic; MTD, maximum tolerated dose; PDAC, pancreatic ductal adenocarcinoma; 5-FU, 5-fluorouracil.

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outcomes depending on the particular therapies, tumor types, and prevailing molecular pathways involved in each specific setting. On the other hand, different antineoplastic therapies can affect the expression of TSPs by tumor and non-neoplastic cells and, in turn, altered production of TSP-1 may be a fundamental part of the mechanism of action of the therapeutic agents. A typical example is the antiangiogenic activity of low-dose metronomic (LDM) chemotherapy (see 2.2), mediated in large part by upregulation of TSP-1 [4] or the anti-proliferative and anti-invasive activity of the VEGFR inhibitor apatinib [5], mediated by down-regulation of TSP-1 in glioma cells.

This review aims at providing an overview of how TSPs are involved in tumor response or resistance to anticancer therapies, focusing on their activity on cancer and TME cells, and discussing their potential as biomarkers of tumor response to therapy. The main focus is on TSP-1, since little is known about the roles of the other TSPs in cancer therapy or as potential biomarkers of tumor response.

2. TSP-1 as a mediator of tumor response to therapy

TSPs can influence the tumor response to therapies in many ways, with various outcomes, and by acting directly on the tumor cells or through the control of the structure, composition, and functional properties of the TME. We review evidence of how TSP-1 affects tumor response to drugs, focusing on its actions on the cancer cells, the tumor vasculature and the immune system (Fig. 1).

2.1. Action on tumor cells

Direct action of TSP-1 on tumor cells can have either a positive or a negative impact on tumor response to therapies.

Positive effects of TSP-1 on the efficacy of therapy. Several studies have pointed to a chemosensitizing activity of TSP-1. Low levels of TSP-1 have been associated with reduced chemotherapy sensitivity in various cancer cell lines [6–8]. Downregulation of TSP-1 expression caused by the drug resistance-associated gene txr1 promoted resistance to paclitaxel or docetaxel by reducing drug-induced tumor cell apoptosis, while exogenous TSP-1 and a CD47 agonist peptide sensitized cells to taxane cytotoxicity [6]. Transcriptional down-regulation of TSP-1 by the txr1 gene inhibited drug-induced apoptosis in gastric cancer cells, promoting resistance to oxaliplatin [9].

TSP-1 upregulation by chemotherapeutic agents can make the tumor more sensitive to subsequent treatments. Upregulation of TSP-1 by 5-fluorouracil (5-FU) pre-treatment enhanced cisplatin's efficacy by increasing apoptosis in head and neck squamous cell carcinomas [10]. Similarly, TSP-1 increased after low-dose cisplatin treatment, and reversed paclitaxel resistance in nasopharyngeal carcinoma [11].

TSPs can also control the response to therapy by affecting cellular senescence. In colorectal and breast cancer models of chemotherapy-induced senescence, TSP-1 was secreted by the senescent cells and prevented senescence escape, maintaining the proliferative arrest of tumor cells. The effect was mediated by TSP-1 interaction with CD47, and loss of CD47 expression induced by p21waf1 downregulation and Myc activation led to senescence escape of a subpopulation of more aggressive cancer cells [12].

Recent studies have shown that TSP-1 can also modulate drug effects by altering the metabolic balance. TSP-1 enhanced the antiproliferative effect of gemcitabine in intrahepatic cholangiocarcinoma in in vitro and in vivo models through an alteration of lipid metabolism, mainly associated with reduced CD36-mediated intake of oleic acid and reduced levels of ATP and ROS in the tumor cells [13].

In an orthotopic model of breast cancer, the increment of TSP-1 expression and downregulation of CD47 after inhibition of the chaperon protein GRP78 prevented resistance to anti-estrogen therapy, associated with increased macrophage infiltrate [14,15].

TSP-1 can also potentiate the tumor response to radiotherapy. In a human melanoma xenograft model, TSP-1 administered before radiotherapy potentiated the antineoplastic effect. This radiopotentiating activity was ascribed to a combination of various mechanisms on tumor and endothelial cells, including reduction in the fraction of radiation-resistant hypoxic tumor cells, antiangiogenic activity, and promotion of radiation-induced apoptosis of tumor endothelial cells [16]. Restoration of TSP-1 expression, suppressed in Myc-amplified medulloblastoma cells, increased the sensitivity to radiotherapy and to the DNA-damaging drug etoposide, via AKT signaling, and this activity was recapitulated by the peptidomimetic ABT-898 [17].

Negative effects of TSP-1 on the efficacy of therapy. TSP-1 can

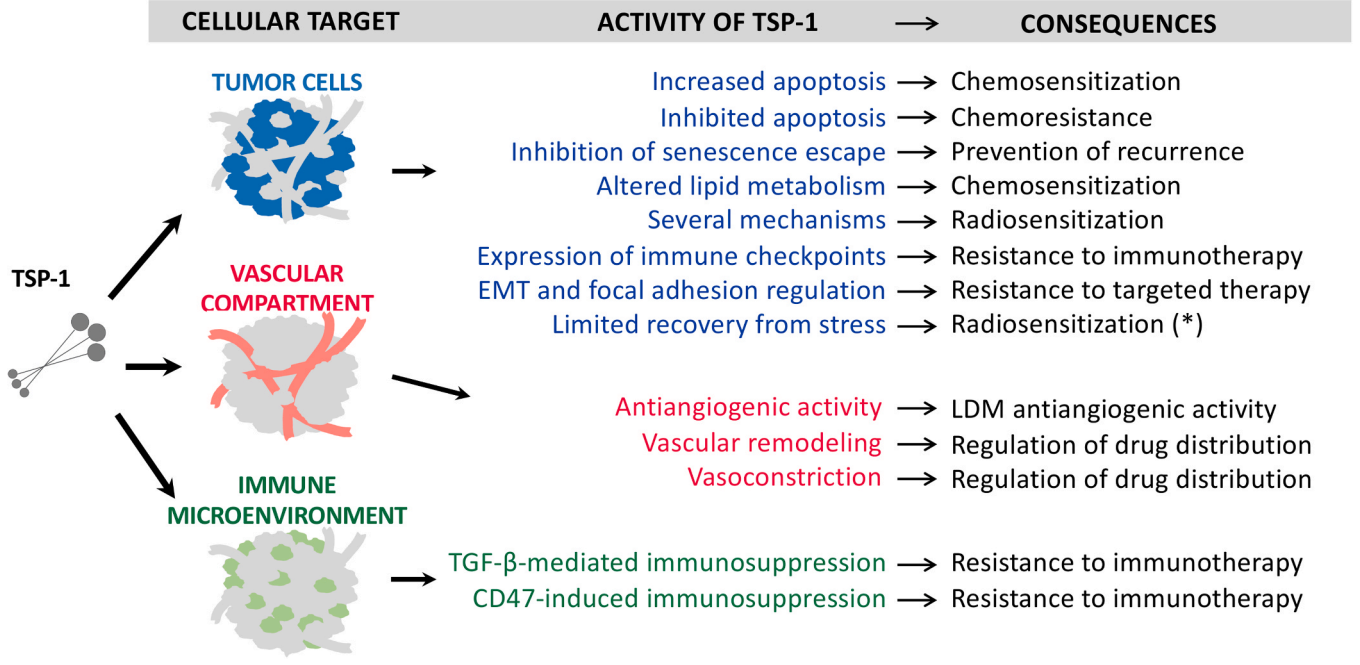

Fig. 1. Schematic representation of how TSP-1 activities on the tumor and TME compartments affect response to therapy. (*) TSP-1/CD47 blockade induces radiosensitization of tumors but protection of normal tissues from radiotoxicity.

negatively influence the response of tumors to antineoplastic therapies, protecting the cells from drug-induced apoptosis. TSP-1 has a protective role against camptothecin- and doxorubicin-induced apoptosis of thyroid carcinoma cells, through interaction with CD47 [18,19]. The two drugs repress TSP-1 synthesis, concomitantly with induction of apoptosis, in a manner dependent on de novo ceramide synthesis and activation of the JNK/ATF-2 pathway [18]. The TSP-1/CD47 interaction blocks JNK phosphorylation, protecting cancer cells from doxorubicin-induced apoptosis, while CD47 blockade boosts JNK activation and sensitizes thyroid carcinoma cells to doxorubicin [19].

Elevated secretion of TSP-1 was associated with resistance to paclitaxel, cisplatin and 5-flurouracil in mesenchymal-like, highly invasive melanoma cells [20]. TSP-1 has been implicated in the resistance of melanoma cells to a BRAF inhibitor [20] and in non-small cell lung cancer resistance to an EGFR inhibitor, though regulation of focal adhesion signaling in the tumor cells [21].

TSP-1 was identified as a key factor responsible for the platelet-induced increase in colon carcinoma cell survival after treatment with paclitaxel [22].

TSP-1's direct action on the tumor cells can also negatively affect their response to immunotherapies (see also 2.3). Inhibition of TSP-1 expression and the resulting reduction of TGF-β activation have been associated with upregulation of tumor expression of PD-L1, lymphocyte infiltration and response to immune checkpoint inhibitors in osteosarcoma [23,24].

The TSP-1/CD47 axis is involved in the complex regulation of cell and tissue response to genotoxic stresses induced by radiotherapy and DNA-damaging drugs. Blockade of CD47 has a protective effect on normal tissues (described in 2.4) but sensitizing activity on tumors. Strategies to block TSP-1/CD47 signaling promote the antineoplastic efficacy of radiotherapy while protecting normal tissues [25]. The antineoplastic activity has been associated with increased recruitment and activation of $CD8^+$ T cells to the tumor site [26,27].

2.2. Action on angiogenesis and the tumor vasculature

TSP-1 is a potent endogenous inhibitor of angiogenesis and modulator of endothelial cell behavior and survival. It affects tumor angiogenesis, vessel perfusion, blood pressure, and remodeling of the tumor vascular network. It is therefore unsurprising that the profound effects of TSPs on the tumor vascular compartment can have a major impact on drug activity.

In line with the antiangiogenic activity of TSP-1, upregulation of TSP-1 synthesis at both the transcriptional and transductional levels has been identified as a major mechanism for the antiangiogenic activity of LDM chemotherapy [4,28]. Bocci et al. demonstrated that TSP-1 was responsible for the particular sensitivity of endothelial cells to low-dose continuous treatment with chemotherapeutic agents [4]. Several LDM cytotoxic drugs including paclitaxel, epothilone B, cyclophosphamide and vinblastine increased TSP-1 expression, independently of their mechanism of action [4].

Following this pioneering study, the upregulation of TSP-1 production as part of the antiangiogenic activity of low-dose chronic chemotherapy, as opposed to the maximum tolerated dose (MTD), has been described in different tumor types and for different chemotherapeutics, in a number of preclinical tumor models, including prostate cancer [4,29], melanoma and lung carcinoma – where TSP-1 increased both in tumors and perivascular stroma cells – [30], ovarian carcinoma [31], breast cancer [32], and pancreatic cancer [31,33]. The role of TSP-1 in the antiangiogenic and antitumor efficacy of LDM chemotherapy was supported by the lack of activity of low-dose cyclophosphamide in mice and tumor cells lacking TSP-1 [30], or in tumor models where LDM cyclophosphamide failed to increase TSP-1 synthesis [34]. These findings led to the proposed use of circulating TSP-1 as a clinical biomarker to monitor patients' response to LDM chemotherapy (see section 3).

Besides chemotherapy, increased production of TSP-1 has been implicated in the antiangiogenic activity of other compounds, such as ceramide analogs [28], dexrazoxane [35], and quercetin [36].

TSP-1 can act on tumor responses to therapies also through its ability to cause tumor vessel remodeling and normalization, favoring drug delivery, hence treatment efficacy. This has been reported with different antiangiogenic domains of TSP-1. The type 1 repeats domain normalized the vasculature of ovarian cancer models and improved the uptake and activity of chemotherapeutics [37]. ABT-510 peptide, a mimetic of this domain, potentiated the distribution of cisplatin and paclitaxel in epithelial ovarian cancer [38]. Similarly, another antiangiogenic domain of TSP-1, the type 3 repeats, caused structural and functional remodeling of the tumor vasculature, increasing the distribution and efficacy of paclitaxel and cisplatin in a model of ovarian cancer [39]. Drug delivery in tumors is also affected by TSP-1's ability to induce vasoconstriction and affect vascular perfusion by inhibiting NO signaling via CD47 [40].

2.3. Action on the immune compartment

TSP-1 has diverse roles in regulating both the innate and adaptive immune response. Multiple activities have been reported on T lymphocytes and cells of the innate response, such as macrophages, myeloid-derived suppressor cells, NK cells, dendritic cells and neutrophils, mainly mediated by its interaction with CD47 [41]. These activities can affect the tumor response to therapies.

TSP-1 induces tolerogenic signals in dendritic cells, and its depletion induces the cytotoxic T lymphocyte response, promoting antitumor activity and improving the effect of a Neu-DNA vaccine in murine tumor models [42].

TSP-1 has been recently identified as a component of the supramolecular attack particles released by cytotoxic T lymphocytes and NK cells, contributing to their cytotoxic activity [43,44]. However, TSP-1 impairs anti-tumor adaptive immune responses mostly by engaging CD47 and by activating TGF-β, a potent immunosuppressive and pro-metastatic cytokine, associated with reduced response to immunotherapy.

TSP-1 secreted by tumor cells induces an immunosuppressive TME in melanoma, while TSP-1 knockdown in cancer cells stimulates the accumulation of $CD8^+$ T cells and reduced Treg enrichment [45]. Silencing TSP-1 enhanced anti-PD-1 immunotherapy efficacy in a triple negative breast cancer mouse model, by increasing lymphocyte infiltration and PD-1 expression on $CD8^+$ tumor infiltrating lymphocytes [24]. The efficacy of immune checkpoint blockade was also boosted by antisense targeting of CD47 [27] or when TSP-1 was inhibited by oridonin, a diterpenoid derivative which can downregulate TSP-1 expression in tumor cells [45]. The co-delivery in liposomes of oridonin with anti-CTLA-4 or anti-PD-1 antibodies enhanced the melanoma response to immunotherapy, by relieving tumor-derived immunosuppression and activating cytotoxic T cells [45].

In multiple myeloma, Runx2 deficient osteoblasts created an immunosuppressive microenvironment through TSP-1-induced activation of TGF-β, resulting in tumor resistance to the proteasome inhibitor bortezomib. In this model, SRI31277, an antagonist of TSP-1-mediated TGF-β activation, restored drug sensitivity, preventing TGF-β immunosuppression, reducing the activation of immunosuppressive myeloid-derived suppressor cells, and restoring the activity of cytotoxic T cells [46].

TSP-1 binding to CD47 has negative effects on $CD8^+$ T cells, as it limits antigen-dependent T cell activation, induces T cell apoptosis and promotes regulatory T cell differentiation [47–50], thus potentially impairing the efficacy of immunotherapies. CD47 and TSP-1 expression in the TME correlate with a weak response to the immune checkpoint inhibitor anti-PD1 in melanoma patients [51]. In a syngeneic model of melanoma, targeting the CD47 signaling pathway potentiated the response to anti-PD1 therapy and inhibition of tumor growth [51].

These findings point to CD47 as a potential target to boost

immunotherapy. However, CD47 is an ubiquitous receptor expressed in various cell types, and its general targeting by antibodies can cause adverse events, such as hematological toxicity. The orthosteric antagonist TAX2 – a cyclic peptide that binds the C-terminal domain of TSP-1 – has been developed to selectively disrupt the TSP-1/CD47 interaction. In ovarian carcinoma xenografts, TAX2 activated anti-tumor immunity by increasing tumor-infiltrating lymphocytes, and synergized with immune checkpoint inhibition, supporting the value of targeting the TSP-1/CD47 interaction to increase the efficacy of immunotherapy [52].

2.4. Action on normal tissues

Besides the tumor cell compartment and the TME, TSPs can also affect the response to drugs of cells in normal tissues, modulating the adverse effects of drugs. For instance, TSP-2 prevented cardiomyocyte apoptosis and matrix disruption caused by doxorubicin, exerting protective activity against doxorubicin-induced cardiomyopathy [53]. In a breast cancer model, autophagy triggered by systemic suppression of CD47 protected cardiac tissue from doxorubicin toxicity [54].

TSP-1 and CD47 limit cell recovery from stresses, including radiation injury. Soft tissues in mice lacking TSP-1 (but not TSP-2) or CD47 were resistant to radiation damage [55]. CD47 blockade protects non-neoplastic cells (vascular endothelial cells, stem cells and immune cells) against radiotherapy-induced genotoxic and oxidative damage [25,55]. This can be mediated by different mechanisms, including inhibition of NO cytoprotective signaling [55], activation of autophagy [56], metabolic regulation in favor of anabolic repair pathways, including DNA damage repair [57], more efficient DNA double-strand break repair [58], and through activity on stem cell renewal [59].

3. TSPs as biomarkers of response to therapy

TSP expression is increased by several chemotherapeutics, administered as LDM (described in 2.2) but also in standard MTD regimens [10,11,18,60], through regulation of transcription factor activation [61,62] or through epigenetic mechanisms, such as TSP-1 promoter hypermethylation [63,64].

There is mounting evidence of the potential of both tumor- and stroma- derived TSPs as biomarkers able to predict treatment response, a still unmet need in the clinical management of cancer patients. TSPs, particularly TSP-1 and TSP-2, have been identified as indicators of pharmacological response in several types of tumor and in different types of target therapy and chemotherapy regimens.

TSP-1 has been proposed as a biomarker in LDM chemotherapy regimens [4]. Upregulation of TSP-1 after LDM chemotherapeutics has been confirmed in a number of in vivo preclinical models (see 2.2), leading to clinical studies to validate TSP-1 as a potential biomarker of response to LDM chemotherapy [65]. Several phase II clinical trials have confirmed the rise of plasma TSP-1 levels in patients treated with LDM chemotherapy, independently of drug's specific mechanism of action. In many cases, TSP-1 upregulation was associated with downregulation of proangiogenic factors, suggesting a transition from a pro-angiogenic to an anti-angiogenic status [65]. For instance, TSP-1 increased and VEGF decreased in the plasma of metastatic colorectal cancer patients receiving continuous infusion of irinotecan, with the largest effects seen with the lowest doses of the drug [66]. TSP-1 was also increased in patients with gastrointestinal cancer treated with metronomic UFT (a combination of a 5-FU prodrug and uracil) and cyclophosphamide plus celecoxib. TSP-1 levels were higher in patients with stable disease than in those with progressive disease, consistent with the role of TSP-1 in the efficacy of LDM chemotherapy [67]. Similarly, plasma levels of TSP-1 were significantly higher in responders than non-responder prostate cancer patients receiving oral LDM vinorelbine and dexamethasone [68]. TSP-1 was also upregulated in children with acute lymphoid leukemia given maintenance therapy with LDM chemotherapy [69].

However, some studies did not confirm the value of TSP-1 as a biomarker of response to LDM chemotherapy. TSP-1 levels were not increased in advanced non-small cell lung cancer patients treated with metronomic oral vinorelbine and presenting evidence of disease stabilization [70]. Similarly, no correlation was found between TSP-1 levels and clinical outcome in ovarian cancer patients treated with bevacizumab plus LDM cyclophosphamide in a phase II trial [71].

Besides LDM chemotherapy, TSP-1 and TSP-2 have been identified as markers of response to other therapies. Several studies suggested TSP-1 as a taxane specific target and marker of sensitivity to taxanes. Docetaxel increased TSP-1 expression in head and neck squamous cell carcinoma cells in vitro [60]. High tumor expression of TSP-1 significantly correlated with treatment outcome in lung adenocarcinoma patients receiving a first-line docetaxel-gemcitabine regimen [7]. This study also indicated that, in line with the role for TSP-1 downregulation in TXR1-mediated resistance to taxane-based chemotherapy, disease control rate, time to tumor progression and overall survival were significantly better in patients with low TXR/high TSP-1 tumor mRNA expression then patients with high TXR/low TSP-1 [7].

Elevated expression of TSP1 in tumors and plasma was associated with gemcitabine sensitivity in PDX models of intrahepatic cholangiocarcinoma, related to TSP-1 inhibition of CD36-mediated oleic acid intake. In intrahepatic cholangiocarcinoma patients TSP-1 levels could predict the response to gemcitabine [13].

Plasma TSP-1 was elevated and associated with resistance to the EGFR tyrosine kinase inhibitor osimertinib in patients with non-small cell lung cancer [21].

Plasma levels of TSP-2 were higher in patients with pancreatic ductal adenocarcinoma (PDAC) than in healthy controls or patients with pancreatitis [72]. Analysis in preclinical orthotopic models of PDAC-PDX demonstrated the stromal origin of TSP-2 and showed that its plasma levels directly paralleled tumor response to gemcitabine plus Nab-paclitaxel, pointing to TSP-2 as a potential biomarker for early detection of PDAC and to monitor response to therapies [72].

TSP-2 has been explored as a putative predictive biomarker of response in other types of treatment. In a phase IV trial in patients with metastatic renal cell carcinoma, basal serum TSP-2 was identified as a promising predictive biomarker for response to second-line treatment with everolimus [73]. However, in situ hybridization revealed no significant association of stromal expression of TSP-2 with treatment outcome and prognosis in patients with metastatic colorectal cancer treated with bevacizumab plus leucovorin [74].

Although the expression of other TSP family members has been reported to be altered in tumors, little is known about their potential value as markers of treatment response. In a bioinformatic analysis of expression of the five TSPs in public databases, TSP-1, TSP-2, TSP-4 and COMP were associated with poor prognosis, and the expression of all five TSPs correlated with macrophage and dendritic cell infiltration, suggesting their possible value as tissue biomarkers of response to immunotherapies [75].

4. Conclusions and future perspectives

The complexity of TSP activity in the regulation of tumor progression and interaction with the TME is reflected by the complex effects, particularly of TSP-1, in tumor response to therapies.

TSP-1 has been reported to affect the tumor response to different therapeutics, with different outcomes (Fig. 1). The intrinsic anti-angiogenic and vascular remodeling activities of TSP-1 are generally beneficial in cancer therapy, potentiating the activity of treatments, improving drug distribution, and acting as a mediator of the anti-angiogenic activity of LDM chemotherapy. TSP-1 typically has immunosuppressive effects, mainly through activation of TGF-β and interaction with CD47 on immune cells, leading to treatment resistance in various cancers. TSP-1/CD47 blockade can restore immune system activity and reverse resistance to immunotherapies. The direct activities of TSP-1 on tumor cells have more heterogeneous final effect on therapy,

mostly depending on the tumor type, the specific receptors and ligands involved, and microenvironmental conditions.

In all cases, the activity of TSPs depends on specific interactions of TSP domains with receptors and ligands. Therefore, efforts to develop TSP-based therapeutic compounds to potentiate the therapeutic efficacy of current therapies have focused on specific active sequences, selectively targeting or mimicking TSP interaction with the specific receptors or ligands.

Synthetic peptides corresponding to antiangiogenic sequences in the type 1 repeats domain, such as ABT-510 [76] and the fusion molecule CVX-045 [77], showed promising antiangiogenic and chemosensitizing activity as single agents and in combination therapies in several pre-clinical models. However, they had no clinical efficacy or showed toxicity in clinical trials [78,79].

Other TSP-1-based approaches that could potentially improve tumor response to drugs are inhibitors of TSP-1-mediated activation of TGF-β [80], or compounds based on the type 3 repeats domain that improve chemotherapy delivery and efficacy [39,81]. The recent finding of the antineoplastic activity of gabapentin, an inhibitor of the TSP-1 neuronal receptor α2δ-1, in an orthotopic model of glioblastoma offers an additional TSP-1-based therapeutic strategy applicable to brain tumors [82].

The possibility of targeting the TSP1/CD47 interaction has attracted considerable interest. Agonist peptides have been developed that mimic the putative CD47-binding sequence of TSP-1, such as PKT16 and PKHB1. PKT16 induced caspase-independent immunogenic cell death in hematological malignancies, in a CD47-dependent manner [83]. Similarly, PKHB1induced cell death in several cancers [84], but in non-small cell lung cancer, it acted in a CD47-independent manner, triggering endoplasmic reticulum stress [85].

Another promising approach to target the TSP-1/CD47 interaction is offered by the TAX2 peptide, designed on the TSP-1-binding sequence of CD47, that has shown antineoplastic and immunostimulating activity in different preclinical cancer models [52].

The development of compounds perturbing or mimicking TSP-1 interactions with specific receptors on tumors and non-neoplastic cells might lead to new strategies to exploit this matricellular protein for approaches to make therapy more effective, improve drug delivery and prevent drug resistance.

Declaration of Competing Interest

None.

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References

[1] S. Kaur, S.M. Bronson, D. Pal-Nath, T.W. Miller, D.R. Soto-Pantoja, D.D. Roberts, Functions of thrombospondin-1 in the tumor microenvironment, Int J. Mol. Sci. 22 (2021) 4570, https://doi.org/10.3390/ijms22094570.

[2] J. Petrik, S. Lauks, B. Garlisi, J. Lawler, Thrombospondins in the tumor microenvironment, 00127–1, Semin Cell Dev. Biol. S1084–9521 (23) (2023), https://doi.org/10.1016/j.semcdb.2023.05.010.

[3] L. Carminati, E. Carlessi, E. Longhi, G. Taraboletti, Controlled extracellular proteolysis of thrombospondins, Matrix Biol. 119 (2023) 82–100, https://doi.org/10.1016/j.matbio.2023.03.011.

[4] G. Bocci, G. Francia, S. Man, J. Lawler, R.S. Kerbel, Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy, Proc. Natl. Acad. Sci. USA 100 (2003) 12917–12922, https://doi.org/10.1073/pnas.2135406100.

[5] H. Yao, J. Liu, C. Zhang, Y. Shao, X. Li, Z. Yu, Y. Huang, Apatinib inhibits glioma cell malignancy in patient-derived orthotopic xenograft mouse model by targeting thrombospondin 1/myosin heavy chain 9 axis, Cell Death Dis. 12 (2021) 927, https://doi.org/10.1038/s41419-021-04225-2.

[6] C.-J. Lih, W. Wei, S.N. Cohen, Txr1: a transcriptional regulator of thrombospondin-1 that modulates cellular sensitivity to taxanes, Genes Dev. 20 (2006) 2082–2095, https://doi.org/10.1101/gad.1441306.

[7] C. Papadaki, D. Mavroudis, M. Trypaki, A. Koutsopoulos, E. Stathopoulos, D. Hatzidaki, E. Tsakalaki, V. Georgoulias, J. Souglakos, Tumoral expression of TXR1 and TSP1 predicts overall survival of patients with lung adenocarcinoma treated with first-line docetaxel-gemcitabine regimen, Clin. Cancer Res 15 (2009) 3827–3833, https://doi.org/10.1158/1078-0432.CCR-08-3027.

[8] S.-L. Li, F. Ye, W.-J. Cai, H.-D. Hu, P. Hu, H. Ren, F.-F. Zhu, D.-Z. Zhang, Quantitative proteome analysis of multidrug resistance in human ovarian cancer cell line, J. Cell Biochem 109 (2010) 625–633, https://doi.org/10.1002/jcb.22413.

[9] J. Bi, Z. Bai, X. Ma, J. Song, Y. Guo, J. Zhao, X. Yi, S. Han, Z. Zhang, Txr1: an important factor in oxaliplatin resistance in gastric cancer, Med Oncol. 31 (2014) 807, https://doi.org/10.1007/s12032-013-0807-1.

[10] B. Xu, P. Liu, 5-FU pretreatment potentiates cisplatin-induced apoptosis through up-regulation of thrombospondin-1 in head and neck squamous cell carcinomas, Cancer Chemother. Pharm. 63 (2009) 1181–1183, https://doi.org/10.1007/s00280-008-0880-x.

[11] X. Peng, W. Li, G. Tan, Reversal of taxol resistance by cisplatin in nasopharyngeal carcinoma by upregulating thromspondin-1 expression, Anticancer Drugs 21 (2010) 381–388, https://doi.org/10.1097/CAD.0b013e3283363980.

[12] J. Guillon, C. Petit, M. Moreau, B. Toutain, C. Henry, H. Roché, N. Bonichon-Lamichhane, J.P. Salmon, J. Lemonnier, M. Campone, V. Verrièle, E. Lelièvre, C. Guette, O. Coqueret, Regulation of senescence escape by TSP1 and CD47 following chemotherapy treatment, Cell Death Dis. 10 (2019) 199, https://doi.org/10.1038/s41419-019-1406-7.

[13] D.-Y. Ding, X.-J. Gan, J.-N. Zhang, G.-J. Hou, Q.-F. Tao, D.-P. Sun, W. Li, Y. Yang, W.-B. Ding, J. Yu, L. Liu, F. Yang, W.-P. Zhou, S.-X. Yuan, Serum thrombospondin-1 serves as a novel biomarker and agonist of gemcitabine-based chemotherapy in intrahepatic cholangiocarcinoma, Br. J. Cancer 128 (2023) 907–917, https://doi.org/10.1038/s41416-022-02101-0.

[14] K.L. Cook, D.R. Soto-Pantoja, P.A.G. Clarke, M.I. Cruz, A. Zwart, A. Wärri, L. Hilakivi-Clarke, D.D. Roberts, R. Clarke, Endoplasmic reticulum stress protein GRP78 modulates lipid metabolism to control drug sensitivity and antitumor immunity in breast cancer, Cancer Res 76 (2016) 5657–5670, https://doi.org/10.1158/0008-5472.CAN-15-2616.

[15] K.L. Cook, D.R. Soto-Pantoja, "UPRegulation" of CD47 by the endoplasmic reticulum stress pathway controls anti-tumor immune responses, Biomark. Res 5 (2017) 26, https://doi.org/10.1186/s40364-017-0105-8.

[16] E.K. Rofstad, K. Henriksen, K. Galappathi, B. Mathiesen, 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 63 (2003) 4055–4061.

[17] T.S.Y. Chan, D. Picard, C.E. Hawkins, M. Lu, S. Pfister, A. Korshunov, M.F. Roussel, R.J. Wechsler-Reya, J. Henkin, E. Bouffet, A. Huang, Thrombospondin-1 mimetics are promising novel therapeutics for MYC-associated medulloblastoma, Neurooncol Adv. 3 (2021) vdab002, https://doi.org/10.1093/noajnl/vdab002.

[18] G. Rath, C. Schneider, B. Langlois, H. Sartelet, H. Morjani, H.E.L. Btaouri, S. Dedieu, L. Martiny, De novo ceramide synthesis is responsible for the anti-tumor properties of camptothecin and doxorubicin in follicular thyroid carcinoma, Int J. Biochem Cell Biol. 41 (2009) 1165–1172, https://doi.org/10.1016/j.biocel.2008.10.021.

[19] M.-P. Courageot, L. Duca, L. Martiny, E. Devarenne-Charpentier, H. Morjani, H. El Btaouri, Thrombospondin-1 receptor CD47 overexpression contributes to P-glycoprotein-mediated multidrug resistance against doxorubicin in thyroid carcinoma FTC-133 Cells, Front Oncol. 10 (2020), 551228, https://doi.org/10.3389/fonc.2020.551228.

[20] A. Jayachandran, M. Anaka, P. Prithviraj, C. Hudson, S.J. McKeown, P.-H. Lo, L. J. Vella, C.R. Goding, J. Cebon, A. Behren, Thrombospondin 1 promotes an aggressive phenotype through epithelial-to-mesenchymal transition in human melanoma, Oncotarget 5 (2014) 5782–5797, https://doi.org/10.18632/oncotarget.2164.

[21] Z. Kosibaty, O.T. Brustugun, I.J. Zwicky Eide, G. Tsakonas, O. Grundberg, L. De Petris, M. McGowan, P. Hydbring, S. Ekman, Ras-related protein Rab-32 and thrombospondin 1 confer resistance to the EGFR Tyrosine kinase inhibitor osimertinib by activating focal adhesion kinase in non-small cell lung cancer, Cancers 14 (2022) 3430, https://doi.org/10.3390/cancers14143430.

[22] A. Radziwon-Balicka, C. Medina, L. O'Driscoll, A. Treumann, D. Bazou, I. Inkielewicz-Stepniak, A. Radomski, H. Jow, M.W. Radomski, Platelets increase survival of adenocarcinoma cells challenged with anticancer drugs: mechanisms and implications for chemoresistance, Br. J. Pharm. 167 (2012) 787–804, https://doi.org/10.1111/j.1476-5381.2012.01991.x.

[23] Z. Liu, J. Wen, F. Hu, J. Wang, C. Hu, W. Zhang, Thrombospondin-1 induced programmed death-ligand 1-mediated immunosuppression by activating the STAT3 pathway in osteosarcoma, Cancer Sci. 113 (2022) 432–445, https://doi.org/10.1111/cas.15237.

[24] E. Marcheteau, T. Farge, M. Pérès, G. Labrousse, J. Tenet, S. Delmas, M. Chusseau, R. Duprez-Paumier, C. Franchet, F. Dalenc, C. Imbert, J. Noujarède, C. Colacios, H. Prats, F. Cabon, B. Ségui, Thrombospondin-1 Silencing Improves Lymphocyte Infiltration in Tumors and Response to Anti-PD-1 in Triple-Negative Breast Cancer, Cancers (Basel) 13 (2021) 4059, https://doi.org/10.3390/cancers13164059.

[25] J.B. Maxhimer, D.R. Soto-Pantoja, L.A. Ridnour, H.B. Shih, W.G. Degraff, M. Tsokos, D.A. Wink, J.S. Isenberg, D.D. Roberts, Radioprotection in normal tissue and delayed tumor growth by blockade of CD47 signaling, Sci. Transl. Med 1 (2009) 3ra7, https://doi.org/10.1126/scitranslmed.3000139.

[26] D.R. Soto-Pantoja, M. Terabe, A. Ghosh, L.A. Ridnour, W.G. DeGraff, D.A. Wink, J. A. Berzofsky, D.D. Roberts, CD47 in the tumor microenvironment limits cooperation between antitumor T-cell immunity and radiotherapy, Cancer Res 74 (2014) 6771–6783, https://doi.org/10.1158/0008-5472.CAN-14-0037-T.
[27] A.L. Schwartz, P.R. Nath, M. Allgauer, E.C. Lessey-Morillon, J.M. Sipes, L. A. Ridnour, Y.M. Morillon Ii, Z. Yu, N.P. Restifo, D.D. Roberts, Antisense targeting of CD47 enhances human cytotoxic T-cell activity and increases survival of mice bearing B16 melanoma when combined with anti-CTLA4 and tumor irradiation, Cancer Immunol. Immunother. 68 (2019) 1805–1817, https://doi.org/10.1007/s00262-019-02397-7.
[28] G. Bocci, A. Fioravanti, P. Orlandi, T. Di Desidero, G. Natale, G. Fanelli, P. Viacava, A.G. Naccarato, G. Francia, R. Danesi, Metronomic ceramide analogs inhibit angiogenesis in pancreatic cancer through up-regulation of caveolin-1 and thrombospondin-1 and down-regulation of cyclin D1, Neoplasia 14 (2012) 833–845, https://doi.org/10.1593/neo.12772.
[29] J.-E. Damber, C. Vallbo, P. Albertsson, B. Lennernäs, K. Norrby, The anti-tumour effect of low-dose continuous chemotherapy may partly be mediated by thrombospondin, Cancer Chemother. Pharm. 58 (2006) 354–360, https://doi.org/10.1007/s00280-005-0163-8.
[30] Y. Hamano, H. Sugimoto, M.A. Soubasakos, M. Kieran, B.R. Olsen, J. Lawler, A. Sudhakar, R. Kalluri, Thrombospondin-1 associated with tumor microenvironment contributes to low-dose cyclophosphamide-mediated endothelial cell apoptosis and tumor growth suppression, Cancer Res 64 (2004) 1570–1574, https://doi.org/10.1158/0008-5472.can-03-3126.
[31] M. Vives, M.M. Ginestà, K. Gracova, M. Graupera, O. Casanovas, G. Capellà, T. Serrano, B. Laquente, F. Viñals, Metronomic chemotherapy following the maximum tolerated dose is an effective anti-tumour therapy affecting angiogenesis, tumour dissemination and cancer stem cells, Int J. Cancer 133 (2013) 2464–2472, https://doi.org/10.1002/ijc.28259.
[32] W.-Y. Tao, X.-S. Liang, Y. Liu, C.-Y. Wang, D. Pang, Decrease of let-7f in low-dose metronomic Paclitaxel chemotherapy contributed to upregulation of thrombospondin-1 in breast cancer, Int J. Biol. Sci. 11 (2015) 48–58, https://doi.org/10.7150/ijbs.9969.
[33] B. Laquente, C. Lacasa, M.M. Ginestà, O. Casanovas, A. Figueras, M. Galán, I. G. Ribas, J.R. Germà, G. Capellà, F. Viñals, Antiangiogenic effect of gemcitabine following metronomic administration in a pancreas cancer model, Mol. Cancer Ther. 7 (2008) 638–647, https://doi.org/10.1158/1535-7163.MCT-07-2122.
[34] L. Jia, D.J. Waxman, Thrombospondin-1 and pigment epithelium-derived factor enhance responsiveness of KM12 colon tumor to metronomic cyclophosphamide but have disparate effects on tumor metastasis, Cancer Lett. 330 (2013) 241–249, https://doi.org/10.1016/j.canlet.2012.11.055.
[35] S.L. Maloney, D.C. Sullivan, S. Suchting, J.M.J. Herbert, E.M. Rabai, Z. Nagy, J. Barker, S. Sundar, R. Bicknell, Induction of thrombospondin-1 partially mediates the anti-angiogenic activity of dexrazoxane, Br. J. Cancer 101 (2009) 957–966, https://doi.org/10.1038/sj.bjc.6605203.
[36] J.P. Yang, Y.D. Liao, D.M. Mai, P. Xie, Y.Y. Qiang, L.S. Zheng, M.Y. Wang, Y. Mei, D.F. Meng, L. Xu, L. Cao, Q. Yang, X.X. Yang, W.B. Wang, L.X. Peng, B.J. Huang, C. N. Qian, Tumor vasculogenic mimicry predicts poor prognosis in cancer patients: a meta-analysis, Angiogenesis 19 (2016) 191–200, https://doi.org/10.1007/s10456-016-9500-2.
[37] S. Russell, M. Duquette, J. Liu, R. Drapkin, J. Lawler, J. Petrik, 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 (2015) 576–588, https://doi.org/10.1096/fj.14-261636.
[38] N.E. Campbell, J. Greenaway, J. Henkin, R.A. Moorehead, J. Petrik, The thrombospondin-1 mimetic ABT-510 increases the uptake and effectiveness of cisplatin and paclitaxel in a mouse model of epithelial ovarian cancer, Neoplasia 12 (2010) 275–283, https://doi.org/10.1593/neo.91880.
[39] D. Pinessi, A. Resovi, F. Sangalli, L. Morosi, L. Zentilin, P. Borsotti, E. Carlessi, A. Passoni, E. Davoli, D. Belotti, R. Giavazzi, M. Giacca, G. Valbusa, A. Berndt, M. Zucchetti, G. Taraboletti, Tumor vascular remodeling by thrombospondin-1 enhances drug delivery and antineoplastic activity, Matrix Biol. 103– 104 (2021) 22–36, https://doi.org/10.1016/j.matbio.2021.10.001.
[40] J.S. Isenberg, F. Hyodo, L.A. Ridnour, C.S. Shannon, D.A. Wink, M.C. Krishna, D. D. Roberts, Thrombospondin 1 and vasoactive agents indirectly alter tumor blood flow, Neoplasia 10 (2008) 886–896, https://doi.org/10.1593/neo.08264.
[41] S. Kaur, D.D. Roberts, Emerging functions of thrombospondin-1 in immunity, Semin Cell Dev. Biol. S1084–9521 (23) (2023) 00117–00119, https://doi.org/10.1016/j.semcdb.2023.05.008.
[42] T.-Y. Weng, S.-S. Huang, M.-C. Yen, C.-C. Lin, Y.-L. Chen, C.-M. Lin, W.-C. Chen, C.-Y. Wang, J.-Y. Chang, M.-D. Lai, A novel cancer therapeutic using thrombospondin 1 in dendritic cells, Mol. Ther. 22 (2014) 292–302, https://doi.org/10.1038/mt.2013.236.
[43] A.R. Ambrose, K.S. Hazime, J.D. Worboys, O. Niembro-Vivanco, D.M. Davis, Synaptic secretion from human natural killer cells is diverse and includes supramolecular attack particles, Proc. Natl. Acad. Sci. USA 117 (2020) 23717–23720, https://doi.org/10.1073/pnas.2010274117.
[44] Š. Bálint, S. Müller, R. Fischer, B.M. Kessler, M. Harkiolaki, S. Valitutti, M. L. Dustin, Supramolecular attack particles are autonomous killing entities released from cytotoxic T cells, Science 368 (2020) 897–901, https://doi.org/10.1126/science.aay9207.
[45] Q. Xiao, X. Li, C. Liu, Y. Jiang, Y. He, W. Zhang, H.S. Azevedo, W. Wu, Y. Xia, W. He, Improving cancer immunotherapy via co-delivering checkpoint blockade and thrombospondin-1 downregulator, Acta Pharm. Sin. B (2022), https://doi.org/10.1016/j.apsb.2022.07.012.
[46] C. Zhang, X. Xu, T.N. Trotter, P.S. Gowda, Y. Lu, M.J. Suto, A. Javed, J.E. Murphy-Ullrich, J. Li, Y. Yang, Runx2 deficiency in osteoblasts promotes myeloma resistance to bortezomib by increasing TSP-1-dependent TGFβ1 activation and suppressing immunity in bone marrow, Mol. Cancer Ther. 21 (2022) 347–358, https://doi.org/10.1158/1535-7163.MCT-21-0310.
[47] P.P. Manna, W.A. Frazier, The mechanism of CD47-dependent killing of T cells: heterotrimeric Gi-dependent inhibition of protein kinase A, J. Immunol. 170 (2003) 3544–3553, https://doi.org/10.4049/jimmunol.170.7.3544.
[48] P. Grimbert, S. Bouguermouh, N. Baba, T. Nakajima, Z. Allakhverdi, D. Braun, H. Saito, M. Rubio, G. Delespesse, M. Sarfati, Thrombospondin/CD47 interaction: a pathway to generate regulatory T cells from human CD4+ CD25- T cells in response to inflammation, J. Immunol. 177 (2006) 3534–3541, https://doi.org/10.4049/jimmunol.177.6.3534.
[49] Z. Li, M.J. Calzada, J.M. Sipes, J.A. Cashel, H.C. Krutzsch, D.S. Annis, D.F. Mosher, D.D. Roberts, Interactions of thrombospondins with alpha4beta1 integrin and CD47 differentially modulate T cell behavior, J. Cell Biol. 157 (2002) 509–519, https://doi.org/10.1083/jcb.200109098.
[50] T.W. Miller, S. Kaur, K. Ivins-O'Keefe, D.D. Roberts, Thrombospondin-1 is a CD47-dependent endogenous inhibitor of hydrogen sulfide signaling in T cell activation, Matrix Biol. 32 (2013) 316–324, https://doi.org/10.1016/j.matbio.2013.02.009.
[51] E.R. Stirling, M. Terabe, A.S. Wilson, M. Kooshki, L.M. Yamaleyeva, M. A. Alexander-Miller, W. Zhang, L.D. Miller, P.L. Triozzi, D.R. Soto-Pantoja, Targeting the CD47/thrombospondin-1 signaling axis regulates immune cell bioenergetics in the tumor microenvironment to potentiate antitumor immune response, J. Immunother. Cancer 10 (2022), e004712, https://doi.org/10.1136/jitc-2022-004712.
[52] A. Jeanne, T. Sarazin, M. Charlé, C. Moali, C. Fichel, C. Boulagnon-Rombi, M. Callewaert, M.-C. Andry, E. Diesis, F. Delolme, D. Rioult, S. Dedieu, Targeting ovarian carcinoma with TSP-1:CD47 antagonist TAX2 activates anti-tumor immunity, Cancers 13 (2021) 5019, https://doi.org/10.3390/cancers13195019.
[53] G.C. van Almen, M. Swinnen, P. Carai, W. Verhesen, J.P.M. Cleutjens, J. D'hooge, F.K. Verheyen, Y.M. Pinto, B. Schroen, P. Carmeliet, S. Heymans, Absence of thrombospondin-2 increases cardiomyocyte damage and matrix disruption in doxorubicin-induced cardiomyopathy, J. Mol. Cell Cardiol. 51 (2011) 318–328, https://doi.org/10.1016/j.yjmcc.2011.05.010.
[54] Y.R. Feliz-Mosquea, A.A. Christensen, A.S. Wilson, B. Westwood, J. Varagic, G. C. Meléndez, A.L. Schwartz, Q.-R. Chen, L. Mathews Griner, R. Guha, C.J. Thomas, M. Ferrer, M.J. Merino, K.L. Cook, D.D. Roberts, D.R. Soto-Pantoja, Combination of anthracyclines and anti-CD47 therapy inhibit invasive breast cancer growth while preventing cardiac toxicity by regulation of autophagy, Breast Cancer Res Treat. 172 (2018) 69–82, https://doi.org/10.1007/s10549-018-4884-x.
[55] J.S. Isenberg, J.B. Maxhimer, F. Hyodo, M.L. Pendrak, L.A. Ridnour, W.G. DeGraff, M. Tsokos, D.A. Wink, D.D. Roberts, Thrombospondin-1 and CD47 limit cell and tissue survival of radiation injury, Am. J. Pathol. 173 (2008) 1100–1112, https://doi.org/10.2353/ajpath.2008.080237.
[56] D.R. Soto-Pantoja, T.W. Miller, M.L. Pendrak, W.G. DeGraff, C. Sullivan, L. A. Ridnour, M. Abu-Asab, D.A. Wink, M. Tsokos, D.D. Roberts, CD47 deficiency confers cell and tissue radioprotection by activation of autophagy, Autophagy 8 (2012) 1628–1642, https://doi.org/10.4161/auto.21562.
[57] T.W. Miller, D.R. Soto-Pantoja, A.L. Schwartz, J.M. Sipes, W.G. DeGraff, L. A. Ridnour, D.A. Wink, D.D. Roberts, CD47 receptor globally regulates metabolic pathways that control resistance to ionizing radiation, J. Biol. Chem. 290 (2015) 24858–24874, https://doi.org/10.1074/jbc.M115.665752.
[58] S. Kaur, A.L. Schwartz, D.G. Jordan, D.R. Soto-Pantoja, B. Kuo, A.G. Elkahloun, L. Mathews Griner, C.J. Thomas, M. Ferrer, A. Thomas, S.-W. Tang, V.N. Rajapakse, Y. Pommier, D.D. Roberts, Identification of Schlafen-11 as a target of CD47 signaling that regulates sensitivity to ionizing radiation and topoisomerase inhibitors, Front Oncol. 9 (2019) 994, https://doi.org/10.3389/fonc.2019.00994.
[59] S. Kaur, D.R. Soto-Pantoja, E.V. Stein, C. Liu, A.G. Elkahloun, M.L. Pendrak, A. Nicolae, S.P. Singh, Z. Nie, D. Levens, J.S. Isenberg, D.D. Roberts, Thrombospondin-1 signaling through CD47 inhibits self-renewal by regulating c-Myc and other stem cell transcription factors, Sci. Rep. 3 (2013) 1673, https://doi.org/10.1038/srep01673.
[60] G.H. Yoo, M.P. Piechocki, J.F. Ensley, T. Nguyen, J. Oliver, H. Meng, D. Kewson, T. Y. Shibuya, F. Lonardo, M.A. Tainsky, Docetaxel induced gene expression patterns in head and neck squamous cell carcinoma using cDNA microarray and PowerBlot, Clin. Cancer Res 8 (2002) 3910–3921.
[61] H.-Y. Zhao, A. Ooyama, M. Yamamoto, R. Ikeda, M. Haraguchi, S. Tabata, T. Furukawa, X.-F. Che, S. Zhang, T. Oka, M. Fukushima, M. Nakagawa, M. Ono, M. Kuwano, S. Akiyama, Molecular basis for the induction of an angiogenesis inhibitor, thrombospondin-1, by 5-fluorouracil, Cancer Res 68 (2008) 7035–7041, https://doi.org/10.1158/0008-5472.CAN-07-6496.
[62] S. Matsushita, R. Ikeda, Y. Nishizawa, X.-F. Che, T. Furukawa, K. Miyadera, S. Tabata, M. Ushiyama, Y. Tajitsu, M. Yamamoto, Y. Takeda, K. Minami, H. Mataki, T. Kanzaki, K. Yamada, T. Kanekura, S.-I. Akiyama, The role of thymidine phosphorylase in the induction of early growth response protein-1 and thrombospondin-1 by 5-fluorouracil in human cancer carcinoma cells, Int J. Oncol. 36 (2010) 1193–1200, https://doi.org/10.3892/ijo_00000602.
[63] Q. Li, N. Ahuja, P.C. Burger, J.P. Issa, Methylation and silencing of the Thrombospondin-1 promoter in human cancer, Oncogene 18 (1999) 3284–3289, https://doi.org/10.1038/sj.onc.1202663.
[64] D.J. Lindner, Y. Wu, R. Haney, B.S. Jacobs, J.P. Fruehauf, R. Tuthill, E.C. Borden, Thrombospondin-1 expression in melanoma is blocked by methylation and targeted reversal by 5-Aza-deoxycytidine suppresses angiogenesis, Matrix Biol. 32 (2013) 123–132, https://doi.org/10.1016/j.matbio.2012.11.010.

[65] G. Natale, G. Bocci, Does metronomic chemotherapy induce tumor angiogenic dormancy? A review of available preclinical and clinical data, Cancer Lett. 432 (2018) 28–37, https://doi.org/10.1016/j.canlet.2018.06.002.
[66] G. Allegrini, A. Falcone, A. Fioravanti, M.T. Barletta, P. Orlandi, F. Loupakis, E. Cerri, G. Masi, A. Di Paolo, R.S. Kerbel, R. Danesi, M. Del Tacca, G. Bocci, A pharmacokinetic and pharmacodynamic study on metronomic irinotecan in metastatic colorectal cancer patients, Br. J. Cancer 98 (2008) 1312–1319, https://doi.org/10.1038/sj.bjc.6604311.
[67] G. Allegrini, T. Di Desidero, M.T. Barletta, A. Fioravanti, P. Orlandi, B. Canu, S. Chericoni, F. Loupakis, A. Di Paolo, G. Masi, A. Fontana, S. Lucchesi, G. Arrighi, M. Giusiani, A. Ciarlo, G. Brandi, R. Danesi, R.S. Kerbel, A. Falcone, G. Bocci, Clinical, pharmacokinetic and pharmacodynamic evaluations of metronomic UFT and cyclophosphamide plus celecoxib in patients with advanced refractory gastrointestinal cancers, Angiogenesis 15 (2012) 275–286, https://doi.org/10.1007/s10456-012-9260-6.
[68] T. Di Desidero, L. Derosa, L. Galli, P. Orlandi, A. Fontana, A. Fioravanti, R. Marconcini, M. Giorgi, B. Campi, A. Saba, S. Lucchesi, R. Felipetto, R. Danesi, G. Francia, G. Allegrini, A. Falcone, G. Bocci, Clinical, pharmacodynamic and pharmacokinetic results of a prospective phase II study on oral metronomic vinorelbine and dexamethasone in castration-resistant prostate cancer patients, Invest N. Drugs 34 (2016) 760–770, https://doi.org/10.1007/s10637-016-0385-0.
[69] N. Andre, S. Cointe, V. Barlogis, L. Arnaud, R. Lacroix, E. Pasquier, F. Dignat-George, G. Michel, F. Sabatier, Maintenance chemotherapy in children with ALL exerts metronomic-like thrombospondin-1 associated anti-endothelial effect, Oncotarget 6 (2015) 23008–23014, https://doi.org/10.18632/oncotarget.3984.
[70] A. Camerini, C. Puccetti, S. Donati, C. Valsuani, M.C. Petrella, G. Tartarelli, P. Puccinelli, D. Amoroso, Metronomic oral vinorelbine as first-line treatment in elderly patients with advanced non-small cell lung cancer: results of a phase II trial (MOVE trial), BMC Cancer 15 (2015) 359, https://doi.org/10.1186/s12885-015-1354-2.
[71] A.A. Garcia, H. Hirte, G. Fleming, D. Yang, D.D. Tsao-Wei, L. Roman, S. Groshen, S. Swenson, F. Markland, D. Gandara, S. Scudder, R. Morgan, H. Chen, H.-J. Lenz, A.M. Oza, Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia, J. Clin. Oncol. 26 (2008) 76–82, https://doi.org/10.1200/JCO.2007.12.1939.
[72] A. Resovi, M.R. Bani, L. Porcu, A. Anastasia, L. Minoli, P. Allavena, P. Cappello, F. Novelli, A. Scarpa, E. Morandi, A. Falanga, V. Torri, G. Taraboletti, D. Belotti, R. Giavazzi, Soluble stroma-related biomarkers of pancreatic cancer, EMBO Mol. Med 10 (2018), e8741, https://doi.org/10.15252/emmm.201708741.
[73] P. Zeuschner, S. Hölters, M. Stöckle, B. Seliger, A. Mueller, H.S. Bachmann, V. Grünwald, D.C. Christoph, A. Stenzl, M.-O. Grimm, F. Brüning, P.J. Goebell, M. Augustin, F. Roos, J. Harde, I. Benz-Rüd, M. Staehler, K. Junker, Thrombospondin-2 and LDH are putative predictive biomarkers for treatment with everolimus in second-line metastatic clear cell renal cell carcinoma (MARC-2 Study), Cancers (Basel) 13 (2021) 2594, https://doi.org/10.3390/cancers13112594.
[74] A.M. Jubb, H.I. Hurwitz, W. Bai, E.B. Holmgren, P. Tobin, A.S. Guerrero, F. Kabbinavar, S.N. Holden, W.F. Novotny, G.D. Frantz, K.J. Hillan, H. Koeppen, Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer, J. Clin. Oncol. 24 (2006) 217–227, https://doi.org/10.1200/JCO.2005.01.5388.
[75] Y. Lu, X. Kong, W. Zhong, M. Hu, C. Li, Diagnostic, therapeutic, and prognostic value of the thrombospondin family in gastric cancer, Front Mol. Biosci. 8 (2021), 647095, https://doi.org/10.3389/fmolb.2021.647095.
[76] J. Greenaway, J. Henkin, J. Lawler, R. Moorehead, J. Petrik, ABT-510 induces tumor cell apoptosis and inhibits ovarian tumor growth in an orthotopic, syngeneic model of epithelial ovarian cancer, Mol. Cancer Ther. 8 (2009) 64–74, https://doi.org/10.1158/1535-7163.MCT-08-0864.
[77] L. Li, T.A. Leedom, J. Do, H. Huang, J. Lai, K. Johnson, T.F. Osothprarop, J. D. Rizzo, V.R. Doppalapudi, C.W. Bradshaw, R.W. Lappe, G. Woodnutt, N.J. Levin, S.R. Pirie-Shepherd, Antitumor efficacy of a thrombospondin 1 mimetic CovX-body, Transl. Oncol. 4 (2011) 249–257, https://doi.org/10.1593/tlo.11136.
[78] L.H. Baker, E.K. Rowinsky, D. Mendelson, R.A. Humerickhouse, R.A. Knight, J. Qian, R.A. Carr, G.B. Gordon, G.D. Demetri, Randomized, phase II study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 in patients with advanced soft tissue sarcoma, J. Clin. Oncol. 26 (2008) 5583–5588, https://doi.org/10.1200/JCO.2008.17.4706.
[79] A. Molckovsky, L.L. Siu, 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. 1 (2008) 20, https://doi.org/10.1186/1756-8722-1-20.
[80] M.J. Suto, V. Gupta, B. Mathew, W. Zhang, M.A. Pallero, J.E. Murphy-Ullrich, Identification of Inhibitors of Thrombospondin 1 Activation of TGF-β, ACS Med Chem. Lett. 11 (2020) 1130–1136, https://doi.org/10.1021/acsmedchemlett.9b00540.
[81] C. Foglieni, K. Pagano, M. Lessi, A. Bugatti, E. Moroni, D. Pinessi, A. Resovi, D. Ribatti, S. Bertini, L. Ragona, F. Bellina, M. Rusnati, G. Colombo, G. Taraboletti, Integrating computational and chemical biology tools in the discovery of antiangiogenic small molecule ligands of FGF2 derived from endogenous inhibitors, Sci. Rep. 6 (2016) 23432, https://doi.org/10.1038/srep23432.
[82] S. Krishna, A. Choudhury, M.B. Keough, K. Seo, L. Ni, S. Kakaizada, A. Lee, A. Aabedi, G. Popova, B. Lipkin, C. Cao, C. Nava Gonzales, R. Sudharshan, A. Egladyous, N. Almeida, Y. Zhang, A.M. Molinaro, H.S. Venkatesh, A.G.S. Daniel, K. Shamardani, J. Hyer, E.F. Chang, A. Findlay, J.J. Phillips, S. Nagarajan, D. R. Raleigh, D. Brang, M. Monje, S.L. Hervey-Jumper, Glioblastoma remodelling of human neural circuits decreases survival, Nature 617 (2023) 599–607, https://doi.org/10.1038/s41586-023-06036-1.
[83] E. Pramil, L. Herbi Bastian, T. Denèfle, F. Nemati, M. Xiao, E. Lardé, K. Maloum, D. Roos-Weil, E. Chapiro, M. Le Garff-Tavernier, F. Davi, D. Decaudin, M. Sarfati, F. Nguyen-Khac, H. Merle-Béral, P. Karoyan, S.A. Susin, Targeting chronic lymphocytic leukemia with N-methylated thrombospondin-1-derived peptides overcomes drug resistance, Blood Adv. 3 (2019) 2920–2933, https://doi.org/10.1182/bloodadvances.2019000350.
[84] K.M. Calvillo-Rodríguez, R. Mendoza-Reveles, L. Gómez-Morales, A.C. Uscanga-Palomeque, P. Karoyan, A.C. Martínez-Torres, C. Rodríguez-Padilla, PKHB1, a thrombospondin-1 peptide mimic, induces anti-tumor effect through immunogenic cell death induction in breast cancer cells, Oncoimmunology 11 (2022) 2054305, https://doi.org/10.1080/2162402X.2022.2054305.
[85] J. Ye, Y. Yao, J. Zhao, L. Cui, G. Lu, Q. Wang, P. Zhang, J. Zhou, Thrombospondin-1 mimic peptide PKHB1 induced endoplasmic reticulum stress-mediated but CD47-independent apoptosis in non-small cell lung cancer, Drug Dev. Res 84 (2023) 238–252, https://doi.org/10.1002/ddr.22028.