E2F-1 Directly Regulates Thrombospondin 1 Expression

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Abstract

Thrombospondin 1 (TSP1) has been shown to play a critical role in inhibiting angiogenesis, resulting in inhibition of tumor growth and metastases. To figure out TSP1’s regulators will lead to reveal its biological function mechanistically. In this study, we show that E2F-1 could activate the transcription of TSP1 by both promoter assays and Northern blot. Analysis of various TSP1 promoter mutant constructs showed that a sequence located −144/−137 up-stream of the transcriptional initiation site, related to the consensus E2F-responsive sequence, is necessary for the activation. In consistence with up-regulation of TSP-1 activity by over-expression of E2F-1, the knockdown of endogenous E2F-1 inhibited TSP-1 promoter activity significantly, implying that E2F-1 mediated regulation of TSP-1 is relevant in vivo. In addition, E2F-1 could also directly bind to the TSP1 promoter region covering −144/−137 region as revealed by ChIP assays. Furthermore, the E2F-1-induced activation of TSP1 gene transcription is suppressed by pRB1 in a dose-dependent manner. Taken together, the results demonstrate that TSP1 is a novel target for E2F1, which might imply that E2F-1 can affect angiogenesis by modulating TSP1 expression.

Introduction

Thrombospondin 1 (TSP1) is a large oligomeric extracellular matrix glycoprotein that mediates cell-cell and cell-matrix interactions by binding cell-surface receptors including integrins, integrin associated protein (IAP/CD47), CD36, heparin sulfate proteoglycans, low density lipoprotein related protein 1 (LRP1), and very low density lipoprotein receptor (VLDLR) in addition to other extracellular matrix proteins, and cytokines [1,2,3,4,5,6,7,8,9,10]. As the first identified naturally occurring angiogenic inhibitor, TSP1 has been shown to play a critical role in inhibiting angiogenesis, resulting in inhibition of tumor growth and metastases [11,12,13,14]. In consistence with its function in anti-angiogenes, TSP1 could inhibit endothelial cells migration in vitro and induce endothelial cells apoptosis in vivo and in vitro [8,9,10,15,16,17,18,19]. In addition, the roles of TSP1 in wound healing, ischemia, heart remodeling, foreign body reaction, intestinal inflammation and synapse formation have been recognized [20,21,22,23]. The roles of TSP1 in tumor progression are closely associated with its regulation by tumor suppressors and oncogenes. Tumor suppressors including p53, PTEN and smad4 up-regulate TSP1 expression, but the oncogenes including c-jun, v-src and c-myc down-regulate TSP1 expression [12,24,25,26]. Recently, the identification of gabapentin receptor α26-1 as a neuronal thrombospondin receptor re-enforces TSP1’s role in promoting CNS (Central nervous system) synaptogenesis [27,28,29]. Targeted overexpression of TSP1 in mice suppressed wound healing and tumorigenesis, while lack of functional TSP1 resulted in increased vascularization of selected tissues and significantly decreased the number of excitatory synapses [15,21,30]. These observations further refined TSP1’s major functions in vivo.

Taking advantage of cell culture system and zebrafish model, we have identified that ELL could serve as a transcriptional factor to directly up-regulate TSP1 expression [31]. ELL was first identified in acute myeloid leukemia as a translocation partner of MLL. Like other MLL translocation products, the chimera MLL-ELL appears to play an important role in leukemogenesis [32,33,34,35,36]. These findings link the TSP1 with another kind of cancer progression—leukemogenesis, unrelated to its role in anti-angiogenesis.

Although the molecular mechanisms of TSP1 in anti-angiogenesis, as well as in tumor suppression, have not been well elucidated, its regulatory factors including either activators or inhibitors seem to be accounted for acting its role. Therefore, searching for more up-stream genes of TSP1 might be helpful for uncovering its roles mechanistically.

In this study, we found that E2F-1 could transactivate TSP1 promoter activity and TSP1 promoter region contains potential E2F-1 binding consensus sequences. Using promoter assays, Northern blot and ChIP (chromatin immunoprecipitation) assays, we identified that E2F-1 could directly transactivate TSP1 expression by binding to its promoter, further expending TSP1’s regulatory factors.

Materials and Methods

Cell line and plasmid construction

HEK 293 and U2OS cells were obtained from ATCC. Both cells were maintained in Dulbecco Modified Eagle Medium (Gibco) with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% CO2.

The cDNA of human E2F1 was amplified by PCR and cloned into the pCGN-HAM (provided by William Tansey), and pCMV-
tag 2C (Stratagene) by PCR. The full length cDNA of human pRB1 was amplified by RT-PCR from cDNA pool of 293 cells and subcloned into pCGN-HAM. Deletion mutants of E2F1 were generated by PCR and cloned into pCGN-HAM, pCMV-tag 2C vectors. The TSP1 promoter (−2033 to +750) luciferase reporter was described previously [31]. Deletion mutants of the TSP1 promoter were generated by restriction endonuclease digestion or PCR and subcloned into the pGL3-Basic vector (Promega). pGL3-ARF was kindly provided by Gordon Peters. pGL3-Cyclin E was kindly provided by Fred Dick. pGL3-Apafl was cloned from total DNA of 293 cells by PCR using primers: 5′-TATCGGATCACTGACTCTCTGGCTGATCACCCCGAGC-3′ and 5′-TATCAGCTTTAATCCAGAACAAAGGAGGAGGTTT-3′.

To accurately map the response region of E2F1 in the TSP1 promoter, four deletion mutants (−413 to −293, −173 to −113) were generated by PCR and subcloned into pGL3-Basic vector at KpnI and XhoI sites. The primers used for PCR were: TSP1(0)-R, 5′-GATACTCGAGGGCAAGGGAGGAGGCCCG-CCGCGCTTTTAAAGGG-3′; TSP1(−413-F, 5′-ATATCCGGAATTCCTGCTGATCACCCCGAGC-3′; TSP1(−293-F, 5′-GATACTCCCTTATCGGATCACTGACTCTCTGGCTGATCACCCCGAGC-3′; TSP1(−173-F, 5′-GATACTCCCTTATCGGATCACTGACTCTCTGGCTGATCACCCCGAGC-3′; TSP1(−113-F, 5′-GATACTCCCTTATCGGATCACTGACTCTCTGGCTGATCACCCCGAGC-3′. To further verify E2F1 binding sites in the promoter of TSP1, on the basis of TSP1-promoter (−173-0), we did fine mapping for TSP1 promoter (−173-0). All the constructs were confirmed by sequencing.

To verify E2F1′s transactivity on TSP1 promoter, an artificial E2F1 construct was generated by PCR to clone potential E2F1 DNA binding domain (aa110–194) into pVP16 vector (Promega) to form a fusion protein with VP16 transactivation domain (VP16-E2F1(110–194)). The mutant of E2F1 deficiency in DNA binding activity (E2F1(E132)) was generated by PCR and cloned into pCMV-tag2C vector.

To confirm whether the knock-down of E2F1 has effect on TSP1 promoter activity, the siRNA vector targeting for human E2F1 was constructed using pSUPER according to instructions [37]. The 19-nt targeting sequence for E2F1 was 5′-TATCGGATCACTGACTCTCTGGCTGATCACCCCGAGC-3′. U2OS cells growing 6-well plate were transfected with the pCMV-Myc empty vector (Clontech), or vectors expressing Myc-ELL. Total RNA was isolated with Trizol reagent (Invitrogen). Electrophoresis, transfer and hybridization were performed as described previously [31]. Briefly, the membrane was probed using synthesized oligos corresponding to human TSP1 (5′-aaagacacatcatctggccat-3′) and human β-actin (5′-ttatgcgtaatgctgcagc-3′) labeled with biotin at the 3′ end. The signal was detected using the North2South Nucleus Labeling and Detection Kit (Pierce). Photography and data analysis were done as described for the Western blot analysis.

Results
E2F1 activates TSP1 promoter reporter effectively

When we performed experiments for verifying the specificity of TSP1 up-regulated by E2F1, we found E2F1 could also up-regulate TSP1 reporter efficiently. In order to define the response elements in TSP1 promoter, initially, we did fine mapping for TSP1 promoter region (−2033/0) promoter activity stimulated by E2F1, indicating a E2F1 repression domain probably located in this region (0–750) (Fig. 1B). Then, we did dose response experiments for E2F1 overexpression on TSP1 promoter activity. Flag empty vector or different dose of Flag-tagged E2F1 expression vector, TSP1(−2033,0) promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells. As shown in Fig. 1C, the TSP1 promoter reporter and Renilla luciferase expression vector were co-transfected into U2OS cells.
promoter activity was steadily up-regulated along with increasing amounts of E2F1 expression vector. The expression of different amounts of Flag-tagged E2F1 was verified by Western blot using anti-flag monoclonal antibody, the amounts of Flag-E2F-1 for transfection were indicated.

Furthermore, to get a clear picture about the importance of E2F-1’s effect on TSP-1 up-regulation, we chose three other well-known E2F-1 targeting genes’ promoter luciferase reporters including ARF, Apaf1 and Cyclin E for evaluation. As judged by luciferase activity measurements, the transactivity of E2F-1 on TSP-1 promoter was lower than that of ARF, but higher than that of Cyclin E, which is similar to that of Apaf-1 (Fig. 1E). In addition, we did further fine domain mapping for the region −413 to 0 bp. The transactivity of E2F-1 transcriptional activity on four TSP1 promoter constructs, 0.2 μg HA tagged E2F-1/per well was used for transfection in 24-well plates. H, the expression of HA-tagged E2F-1 was verified by Western blot using anti-HA monoclonal antibody.

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Figure 1. E2F-1 activates TSP1 promoter. A, schematic depiction of six TSP1 promoter deletion constructs in the region −2033 to +750bp. The transcription initial site is designated as +1. B, quantification of E2F-1 transcriptional activity on six TSP1 promoter constructs. 293 cells were used for assays and 0.2 μg HA tagged E2F-1/per well was used for transfection in 24-well plates. C, dose response of E2F1 on TSP1 promoter activity in U2OS cells. D, the expression of different doses of Flag-tagged E2F1 was verified by Western blot using anti-flag monoclonal antibody, the amounts of Flag-E2F-1 for transfection were indicated. E, the promoter luciferase reporters of E2F1 targeted gene ARF, Apaf1 and CyclinE were used as controls to evaluate the transactivity of E2F-1 on TSP1 promoter, 0.2 μg HA tagged E2F-1/per well was used for transfection in 24-well plates. F, schematic depiction of four TSP1 promoter deletion constructs in the region −413 to 0 bp. G, quantification of E2F-1 transcriptional activity on four TSP1 promoter constructs. 0.2 μg HA tagged E2F-1/per well was used for transfection in 24-well plates. H, the expression of HA-tagged E2F-1 was verified by Western blot using anti-HA monoclonal antibody.

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As a transcription activator, E2F1 can bind DNA cooperatively with DP proteins through the E2 recognition site (5′-TTTC[CG][CGC]-3′) located in the promoter region of a number of genes whose products are involved in cell cycle regulation or in DNA replication [39]. After searching for the consensus sequences
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-393 ATCAACCAGG CATTCCGGA GATCAGCTCG CCGGAAAGCC CCTGCAGCCAC CCCGCCGGCC
-333 CTCTTAGGGTG GTCTCCCCAG CCCGTCTCTT TTCGCGATAG CTTGCGTATC ACCCCCAGGCC
-273 CGCGTGGCCG AAGATGACGA GCGCCGAGAC CGTCGCGCCG AAATCGTCTGCT GGGCGGGC
-213 CGACTTTTCT GAGAAATTTCT AGTGCTCCCA AGCCCGGAC CCAGCGCCCCT TCACTTTTCT
-153 GCTGGAAGT TGCCGCGGAG GCACGGGGGGG GCAGGAGAGAG AGGCCGAGAG ATGCAGAGGC
-93 CTCCGGCTTC GTGACGGGCC GCGCCGATT GCCGCGAGGA ATCCCCAGGA ATGCGAGGCC
-33 CCCTTTAAAA GCGCGGGGCT CCTCCGCGTT GCCACCGCT GTGCCGTCGA

B

C

D

E

F

G

H

I

J

K

L

Relative luciferase activity

Flag empty
Flag-E2F1

Relative luciferase activity

Flag-E2F1

Relative luciferase activity

Flag-E2F1

Relative luciferase activity

Flag-E2F1

Relative luciferase activity

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Relative luciferase activity

Flag-E2F1
E2F1 Regulates TSP1

**Figure 2. E2F1 binding site in TSP1 promoter is required for E2F1 up-regulation.** A, the partial sequence of TSP1 promoter (−393 to +276bp). Three potential E2F1 binding sites were marked by boxes and substitution mutations in those sites were indicated underline. B, schematic of eight TSP1 promoter mutation constructs in the region −393 to 0bp. The three potential E2F1 binding sites were marked by dark circles and mutations were marked by "X". C, quantification of E2F1 transcriptional activity on eight TSP1 promoter mutation constructs. 0.2 μg Flag tagged E2F1/per well was used for transfection in 24-well plates. D, schematic depiction of E2F1 DNA binding region (amino acid 110–194) cloned into pVP16 vector. E, quantification of E2F1 transcriptional activity on three TSP1 promoter mutants, 0.2 μg VP16-E2F1(110–194) or VP16/per well was used for transfection in 24-well plates. F, quantification of wild-type E2F1 and E2F1(E132) transcriptional activity on TSP1 promoter (−2033–0) luciferase reporter, 0.2 μg vector/per well was used for transfection. G, the expression of HA-tagged wild-type E2F1 and E2F1 DNA binding site mutant (E132) was verified by Western blot using anti-Flag monoclonal antibody. H, quantification of pRB influence on E2F1 transcriptional activity on TSP1 promoter (−2033–0) luciferase reporter construct. The expression of HA-pRB was verified by Western blot and the amounts of HA-pRB for transfection were indicated. I, quantification of E2F1 deletion mutants on TSP1 promoter (−2033–0) luciferase reporter construct. J, the expression of flag-tagged E2F1 deletion constructs was verified by Western blot using anti-Flag monoclonal antibody. 0.2 μg different construct/per well was used for transfection. K, quantification of E2F1 siRNA on TSP1 promoter (−2033–0) luciferase reporter activity. L, the knockdown of E2F1 by siRNA in U2OS cells was verified by Western blot using anti-E2F1 monoclonal antibody, a siRNA targeting GFP was used as control, 0.6 μg siRNA expression vector/per well targeting E2F1 or GFP was used for transfection in 6-well plates.

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for E2F1 binding, we found three potential binding sites presented in TSP1 promoter region between −363 to −137 (Fig 2A). In order to determine whether these E2F1 binding sites in TSP1 promoter were responsible for E2F1 up-regulation, we made substitution mutations for the region −393 to 0 of TSP1 promoter (Fig 2B). Then, Flag empty vector or Flag-tagged E2F1 (0.2 μg/per well in 24-well plates) was co-transfected into 293 cells along with mutant constructs and Renilla. The results from promoter assays showed that −363-mut and −238-mut could partially reverse the transcription activity of E2F1, but −140-mut almost completely reverse the transactivation of E2F1 (Fig 2C).

When the three putative binding sites were mutated simultaneously, the transactivation of TSP1 induced by E2F1 was abolished completely. In addition, the short mutant −140-mut2 also lost its responding for E2F1 up-regulation, further confirming that the consensus binding site located between −144 to −137 was critical for E2F1 responding.

To further test whether E2F1 DNA binding domain was critical for transactivating TSP1, the potential DNA binding domain (110–194 amino acids) was fused with VP16 activation domain using pVP16 vector (Clontech). Subsequently, we co-transfected VP16-E2F1 (110–194) (0.2 μg/per well in 24-well plates) along with TSP1 promoter luciferase reporters TSP1 (−2033 to 0), TSP1(−173 to 0) or TSP1 (−140-mut2) respectively. The luciferase assays showed that VP16-E2F1-(110–194) could indeed transactivate the TSP1 promoter reporters significantly (p<0.0001) but not TSP1 (−140-mut2) (Fig 2E). Moreover, the E2F1 mutant (E132) lacking DNA binding activity failed to transactivate TSP1 promoter (−2033 to 0) (Fig 2F). These observations suggested that the DNA binding activity of E2F1 is required for inducing TSP1 expression. The expression of Flag-tagged wild-type E2F1 and the mutant (E132) were verified by Western blot (Fig 2G) using anti-Flag antibody. In addition, in order to test whether pRB1 could affect the transactivation of E2F1 on TSP1 promoter, we transfected two different dosage of HA-tagged pRB1 (0.2μg and 0.5μg/per well in 24-well plates) together with HA-tagged E2F1(0.2 μg/per well in 24-well plates) and performed promoter assays, pRB1 could indeed suppress the transactivity of E2F1 on TSP1 promoter in a dose-dependent manner (Fig 2H), which is consistent with the effect of pRB1 on other bona fide targets of E2F1. At the same time, different function domains of E2F1 were cloned into Flag-tagged vector and co-transfected into 293 cells with TSP1 promoter luciferase reporter TSP1(−2033 to 0). The luciferase assay showed that only full length E2F1 can up-regulate TSP1 (−2033 to 0) (Fig 2I). The expression of different function domains of E2F1 were verified by Western blot (Fig 2J).

To figure out whether endogenous E2F1 could still have some influence on TSP1 promoter activity, we knocked down the expression of endogenous E2F1 by an E2F1-specific short interfering RNA (siRNA) vector into U2OS cells. As expected, compared to the control (cells transfected with a siRNA vector specifically targeting GFP expression), E2F1 knockdown could inhibit TSP1 promoter (−2033, 0) activity significantly (p = 0.0001) (Fig 2K). The knockdown of endogenous E2F1 was confirmed by Western blot (Fig 2L). The observations suggested that E2F1-mediated regulation of TSP-1 is physiologic relevant.

E2F1 binds to the TSP1 promoter in vivo

To demonstrate whether the action of E2F1 on TSP1 occurs in vivo, we conducted chromatin Immunoprecipitation assay to test the binding of E2F1 in the TSP1 promoter. As shown in Fig 3B, an enrichment of the TSP1 promoter was detected using anti-E2F1 antibody in 293 cells. No signal was observed using a negative control antibody (normal mouse IgG). Primers specific for β-actin was used as control. These results indicated that E2F1 could bind to the TSP1 promoter directly in 293 cells.

E2F1 up-regulates TSP1 mRNA expression

We next want to test whether E2F1 could up-regulate expression of the endogenous TSP1 gene. Total RNA was extracted from 293 cells 24h after transfected by either HA-E2F1 expression vector or equivalent control empty vector. Up-regulation of TSP1 mRNA by E2F1 was confirmed by Northern blot analysis (Fig 4) which suggested that E2F1 could indeed induce TSP1 mRNA expression.

**Discussion**

In this study, through domain mapping for TSP1 promoter, we identified that the E2F1 binding consensus sequence located in −144 to −137 of TSP1 promoter is critical for E2F1 up-regulation. Further ChIP assays confirmed that E2F1 could bind the promoter region covering −144 to −137 in vivo. These observations suggested that E2F1 could regulate TSP1 expression by directly binding to its promoter. In addition, we verified that the DNA binding domain and DNA binding ability of E2F1 were required for transactivating TSP1 expression. Furthermore, we found that the knockdown of endogenous E2F1 could inhibit TSP1 promoter activity significantly, confirming the relevance of E2F1-mediated regulation of TSP1 in vivo. Taken together, these results suggested that TSP1 is a direct target of E2F1.

E2F1 belongs to a large family of transcription factors containing one or more conserved DNA binding domains (DBDs) that bind target promoters and regulate their expression [40,41,42]. To elucidate E2F1’s function, searching for E2F1’s other bona fide targets has been extensively conducted. Through global gene expression profiling and promoter occupancy arrays, many
target genes of E2F-1 being critical for proper cell cycle progression have been identified, which established a direct role for E2F-1 in governing cell proliferation [41,43,44]. Moreover, the roles of E2F-1 in cancer have been well-recognized due to its functions in tumor suppression [43,44,45,46]. Functional inactivation of pRB1 in various human cancers leads to deregulated E2F1 expression, either inhibit its transactivation or enhance its transrepression activity [46]. Here, we presented data to show that pRB1 could also supress E2F-1’s transactivity on TSP1 promoter in a dose-dependent manner, which is consistent with the role of pRB1 in modulating expression of E2F-1 targets, further confirming that TSP1 serves as a bona fide target of E2F-1.

The role of genes in anti-angiogenesis represents a major aspect for their function in tumor suppression [47]. Even though, the roles of E2F-1 in tumor progression have been extensively investigated, however, to date, little is known about its function relevant to angiogenesis. TSP1 is the first identified naturally occurring angiogenic inhibitor, its role in inhibiting angiogenesis, resulting in inhibition of tumor growth and metastases has been well-defined [11], and therefore, the identification of TSP1 as a direct target of E2F-1 might open a new window for demonstrating the role of E2F-1 on anti-angiogenesis, relevant to its function in tumor suppression. Of cause, how E2F-1 acting its role in anti-angiogenesis through the regulation of TSP1 expression is definitely worth to be further investigated.

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Author Contributions

Conceived and designed the experiments: WJ WX. Performed the experiments: WJ WX. Analyzed the data: WJ WX. Contributed reagents/materials/analysis tools: WJ WX. Wrote the paper: WJ WX.

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