Journal of Life Science 2005, Vol. 15. No. 6. 895~903 ©JLS

Helixor A Inhibits Angiogenesis in vitro Via Upregulation of Thrombospondin-1

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Received October 18, 2005 / Accepted November 22, 2005

Thrombospondin-1 (TSP-1), a negative regulator in tumor growth and angiogenesis, is cell-type specifically regulated under pathological conditions or by extracellular stimuli, and the regulation of TSP-1 gene expression is important for developing new approaches in tumor therapy. Mistletoe is a parasitic plant that have been used for immunomodulation and antitumor therapy. Helixor A is an aqueous part of mistletoes extract. Here we showed that TSP-1 expression was significantly induced at both mRNA and protein levels in the Hepatocarcinoma cell line (Hep3B) and primary bovine endothelial cell line (BAE) exposed to Helixor A. Our promoter analysis confirmed that the expression of TSP-1 gene was regulated by Helixor A at the transcriptional level. In cell invasion assay, the conditioned media obtained from treatment of these cells significantly reduced the number of invasive cells and also inhibited capillary-like tube formation of BAE cells on Matrigel. Moreover, the inhibitory effects of the conditioned media on cell invasion and tube formation were reversed by blocking with anti-TSP-1 neutralizing antibodies, suggesting that TSP-1 is involved in Helixor A-induced antiangiogenic effect. Taken together, our results suggest that Helixor A have an antiangiogenic effects through upregulation of TSP-1.

Key words – thrombospondin-1 (TSP-1), Helixor A, angiogenesis

New blood vessel formation (angiogenesis) is an essential process in various physiological and pathological conditions including wound healing, tissue remodeling, chronic inflammatory disease and tumorigenesis[6]. The angiogenic processes are tightly regulated by a balance between negative and positive regulators during formation of blood vessel[7]. Unbalance between negative and positive factors for angiogenesis is considered a hallmark of the malignant process whereby proangiogenic mechanism overwhelm or circumvent negative regulators of angiognenesis[8].

The negative regulators of angiogenesis includes thromobospondin-1 (TSP-1), angiostatin and endostatin. Among them, TSP-1 was first discovered in 1971 as a 450 kDa protein that is released from thrombin-stimulated platelets[1]. Although the effects of TSP-1 on tumorigenesis and angiogenesis are controversial, an increasing evidence has shown that TSP-1 acts as a negative regulator in solid tumor progression and neovascularization[10,11,19]. For example, transfer of TSP-1 cDNA into a human breast carcinoma cell line suppresses primary tumor proliferation, metastatic potential, and angiogenesis[15].

Helixor A is an aqueous extract containing lectins, viscotoxins, and alkaloids isolated from fresh mistletoe growing on different host trees like apple, oak, and pine[2]. Recently, an interesting report has documented that mistletoe extracts repressed tumor proliferation and angiogenesis[20]. Hence, mistletoe extracts is used for all types of cancer, as either palliative treatment for a better quality of life, or as an adjuvant therapy for prevention of relapse. It is also used in combination with traditional chemotherapy where its therapeutic goal is a better tolerance of chemotherapeuic drugs, and a reduction in bone marrow toxicity[17]. The mode of action, is immuno-modulation at low doses, and inhibition of cancer by apoptosis at high doses[3]. Specific effects of mistletoe extracts on the immune system and its actual mechanism underlying the suppression of tumor growth have so far not been unequivocally elucidated.

In present study, we tested the possibility that anti-tumor activity induced by Helixor A could be mediated via upregulation of TSP-1 expression in hepatocarcinoma cell line (Hep3B) and bovine endothelial cell line (BAE). We found that Helixor A remarkably induced the expression of TSP-1 at both mRNA and protein levels via activation of TSP-1 promoter. The increased level of TSP-1 by Helixor A treatment inhibited cell invasion in both cells, and tube formation in BAE cells, which is confirmed by blocking the antiangiogenic action of TSP-1. Therefore, these results pro-

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vide data that TSP-1 acts as an important negative mediator in the Helixor A-induced antiangiogenesis.

Materials and Methods

Materials

Minimum Essential Media (MEM) were purchased from Gibco BRL (Grand Island, NY). LipofectAMINE was purchased from Invitrogen (Rockville, MD). Helixor A was obtained from Boryung Co. (Seoul, Korea) and diluted to a 50 mg/ml stock before use. 3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl-tetrazolium bromide (MTT) were from Sigma (St Louis, MO). TSP-1 antibodies were from NeoMarkers (Fremont, CA). The ECL western blotting detection kit was from Amersham Biosciences (Arlington Height, Ill). Matrigel was purchased from BD Biosciences (Palo Alto, CA). Transwell was purchased from Costar (Cambridge, MA).

Cell culture and Helixor A Treatment

BAE cells (passage 8~14) were cultured in MEM supplemented with 5% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/ml), streptomycin (100 μg/ml), and 20 mM HEPES. Human hepatocarcinoma cell line, Hep3B cells were grown and maintained in Dubecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated FBS and antibiotics. Cells were maintained in a humidified incubator at 37℃ with 5% CO_2. For Northern or Western blot analysis, cells were seeded in a 35-mM diameter-dishes at a density of 1.5×10^5 cells/ml for 24 h, and further cultured in the serum-free media for 16 h. Cells then were treated for various time periods with varying concentrations of Helixor A dissolved in DMSO.

Concentration of conditioned medium

Cell-free supernatant obtained from serum-free culture media was concentrated as previously described with minor modifications[12]. Briefly, cells at 80% confluency were serum-starved for 24 h, and treated with or not Helixor A. At various time points, the cultured media were collected, centrifuged (5000×*g*, 15 min, 4℃) to remove cell debris, and were concentrated up to 5-fold by using a centricon membrane (MW 50.000 cutoff, Amicon, Lexington, MA). The concentrated samples were stored at -70℃ before use.

Northern blot analysis

Total RNA from confluent cells was extracted using STAT-60 (Tel-Test Inc., Friendswood, Tex). RNA samples were separated by electrophoresis in 1% formaldehyde-agarose gels. The RNA was transferred in 20×SSC onto nylon membrane (Schleicher and Shuell, Dassel) by capillary action and UV cross-linked with a Stratalinker UV light source (Stratagene, La Jolla, CA). TSP-1 probe was the DIG-11-dUTP labeled cDNA generated by standard PCR method (Boehringer Mannheim, Mannheim). Blots were prehybridized at 55℃ for 1 h and incubated with the hybridization solution containing the DIG-labeled TSP-1 probe at 55℃ overnight. The Blots were then washed twice in 2×SSC, 0.1 % SDS at room temperature and then twice in 0.1×SSC, 0.1 % SDS at 60℃. The membrane was developed by an immunochemical method using DIG chemiluminescent detection kit (Boehringer Mannheim, Mannheim).

Western blot analysis

Protein contents were quantified by the bicinchronic acid method using BCA protein assay reagent (Pierce, Rockford, IL). Aliquots of the 20 μg of protein solution were boiled in 1 X Laemmli sample buffer and separated on 8% SDS-PAGE. After the migrated proteins were electrophoretically transferred to nitrocellulose membrane (Schleicher and Shuell, Dassel), the membrane was blocked by incubating with 5% nonfat dried milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) overnight at 4℃. The membrane was probed with monoclonal anti-TSP-1 mouse antibody diluted 1:1000 in TBS containing 3% nonfat dried milk for 1 h at room temperature, and then washed three times with TBS-T. The washed membrane was incubated with 1:1000 dilution of peroxidase-conjugated anti-mouse immunoglobulin antibody (Sigma, St Louis, MO) in TBS containing 3% dried nonfat milk for 1 h at room temperature. The antisera-treated membrane was washed three times with TBS-T and immunocomplex was visualized by enhanced chemiluminescence using the ECL kit.

Cytotoxicity Assay

Cell cytotoxicity was determined by MTT assay. Cells were seeded at varied density in a 96-well plate culture overnight and serum-starved for 24 h. Then, dose-dependency of Helixor A was examined. A final media volume were adjusted to 200 μl. After incubation of 24 h, 200 ng/㎖ MTT reagent of final concentration was added to each well. Three hours later, MTT crystals were dissolved with

DMSO. The liquid layer was collected and its absorbance was measured at 490 nm.

Construction of reporter plasmid containing TSP-1 promoter

The 2774 bp TSP-1 promoter region (-2200 to +754) was isolated by PCR with rTth DNA polymerase, XL (PE Applied Biosystems, Foster city, CA) from genomic DNA of Hep3B cells using the following primers : forward primer 5'-GAGGAAGGGCTTTGTGTTTGAGATA-3' and reverse primer 5'-GATCCTGTAGCAGGAAGCACAAG-3'. To generate restriction enzyme sites, the forward primer with *Kpn I* site and reverse primer with *Xho I* site at each 5' end, respectively, was commercially synthesized (Bioneer, Inc.,). PCR was performed for 30 cycles with a condition: 94℃ for 5 min, 94℃ for 30 sec, 50℃ for 30 sec, 72℃ for 3 min, followed by a final extension step at 72℃ for 5 min. Amplified PCR-product was digested with *Kpn I* and *Xho I*. The resulting fragment was then introduced in front of a luciferase gene of the pGL3 Basic vector cleaved with *Kpn I* and *Xho I* (Promega, Madison, WI).

Transient transfection and Luciferase assays

Cells were seeded in 12-well plates at 1×10^5 cells/well, and incubated for 24 h. Prior to transfection, the cells were cultured in 0.4 ml of Opti-MEM (Gibco BRL). DNA constructs (1 μg) in 50 μl Opti-MEM solution were incubated with 5 μl of Plus reagent (Invitrogen, Carlsbad, California) for 15 min at room temperature. Following 15 min incubation at room temperature, 50 μl Opti-MEM solution containing 3 μl LipofectAMINE (Invitrogen, Carlsbad, California) was then added to the solution of DNA constructs-Plus reagent complexes, and the mixture was incubated at room temperature for an additional 30 min. Then, each well was exposed to the DNA mixture, and incubated at 37℃ for 4 h. One milliliter of the media containing 10% FBS was then added to each well and the cells were incubated overnight at 37℃. The next day, the transfected cells were treated with 200 ng/ml Helixor A and incubated for 24 h prior to the determination of promoter activty. Cell lysates were prepared by extraction with 100 μl of reporter lysis buffer (Promega, Madison, WI). Luciferase (LUC) activity was measured using a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). The luciferase activity was normalized by protein concentration for transfection efficiency.

Chemotactic invasion assay

The migration and invasion of the cells were performed *in vitro* using a 48-well Boyden chamber system with 8 μm -pore polycarbonate filter inserts. The lower side of the filter was coated with 10 μl of type I collagen (0.5 mg/ml) and air dried for 1 h. The upper side was coated with 10 μl Matrigel (0.5 mg/ml). Cells (1×10^5 cells/ml) and conditioned media (50~100 μl) were placed in the upper part of the filters. In oder to examine the effects of TSP-1, TSP-1 neutralizing antibodies (Ab-9) were placed in the upper compartment. To confirm whether the activity of TSP-1 antibodies were generated by nonspecific effect, mouse IgG antibodies were also used here. The cells were incubated at 37℃ for 8 h or 12 h and then fixed with methanol followed by staining with hematoxylin. The invasion and migration of cells were determined by counting the whole cell number in a single filter using optical microscopy at a × 40 magnification. Each sample was assayed in duplicate and the experiment was repeated twice independently.

Capillary-like tube formation assay

100 μl of Matrigel (10 mg/ml) was polymerized for 1 h at 37℃. Cells were trypsinized and plated into Matrigel-coated 48-well plates with a cell density of 1×10^5 cells/ml in serum-free MEM medium. Then, conditioned media (50 ~100 μl) were added and incubated for 12 h. To neutralize the TSP-1 effect on the capillary-like tube formation, the specific TSP-1 antiserum (Ab-9) was added to the conditioned media treated cells. In addition, nonspecific mouse IgG antibodies were also used to test whether the inhibiting effect on tube formation were caused by TSP-1 induced specific activity. Morphologic changes of cells were photographed at × 40 magnification.

Statistical analysis

Data are presented as mean values ± S.D. and P values were determined using unpaired Student's *t* test. $P< 0.05$ was accepted as statistically significant.

Results

Helixor A has no cytotoxic effect on BAE and Hep3B cells

We tested whether Helixor A affected cell viability by its cytotoxicity. Helixor A at various concentrations was applied to the both cells and the its cytotoxicity was meas

ured by MTT assay. Helixor A exerted no effect on the viability of BAE cells (Fig. 1A) and Hep3B cells (Fig. 1B) for 24 h at any doses tested. Therefore, the optimal conditions were set to be at 5~500 ng/ml and for 24 h. All experiments were performed in this conditions hereafter.

Helixor A increases TSP-1 expression in BAE and Hep3B cells

Since it has not been reported that Helixor A regulated the expression of TSP-1, we first examined the effect of Helixor A on the expression of TSP-1 in primary bovine aortic endothelial and human hepatocarcinoma cells. Cells were treated with Helixor A at the indicated concentrations and time periods, respectively, as described in Fig 1. The levels of TSP-1 mRNA and protein were determined by Northern Blot and Western analysis, respectively. Helixor A enhanced the TSP-1 expression in a time dependent manner; TSP-1 mRNA started to increase at 3 h and reached a maximum level at 12 h, and thereafter was sustained at the significant amount for 24 h in BAE cells and Hep3B cells (Fig. 2A). Because TSP-1 protein is synthesized and secreted into extracellular environment, the secreted form of TSP-1 was analyzed using the supernatant obtained from defined culture media (CM). In a similar to mRNA kinetics, TSP-1 protein began to increase within 6 h and reached its peak level at 24 h in Helixor A-treated culture media (Fig. 2B). These results indicated that Helixor A increased the synthesis and secretion of TSP-1 protein in BAE cells and Hep3B cells.

Helixor A increases TSP-1 expression via the activation of TSP-1 promoter in BAE and Hep3B cells

The regulation of TSP-1 expression has been demonstrated to be occnred predominantly at transcriptional level. Thus, we investigated the effect of Helixor A on the activity of TSP-1 promoter. The promoter region (-2200~+754) of the TSP-1 gene from genomic DNA of Hep3B cells were isolated, and it was cloned into *Xho I* and *Kpn I* sites on promoterless pGL3 Basic plasmid, which is upstream of the luciferase report gene. The constructs of TSP-1 promoter was transiently transfected into BAE cells (Fig. 3A) and Hep3B cells (Fig. 3B), and determined luciferase activities. Both cells transfected with pTSP-2200 repoter construct showed 2-fold activation by Helixor A as compared to its control, pGL3 basic backbone plasmid. These results are in parallel with other studies that TSP-1 gene expression was mainly controlled through the transcriptional regulation[12].

TSP-1 upregulation by Helixor A inhibits cell invasion in BAE and Hep3B cell

To investigate the effect of Helixor A-induced TSP-1 on cell invasion prerequisite process for angiogenesis, the chemokinetic invasion models were used. We carried out cell invasion test using a modified Boyden chamber invasion assay. BAE cells (Fig. 4A and B) and Hep3B cells (Fig. 4C) suspensions were loaded into upper chamber and immediately treated with each of the cell-free conditioned media cultured with BAE and Hep3B cells for 24 h. Then, the Transwell chamber was incubated at 37℃ for 8-10 h. After the incubation to allow cells to migrate into outer membrane of upper chamber basement, the basement membrane was stained with hematoxylin dye and the number of migrated cells was counted. As shown in Fig. 4. Helixor-A treated conditioned media remarkably reduced

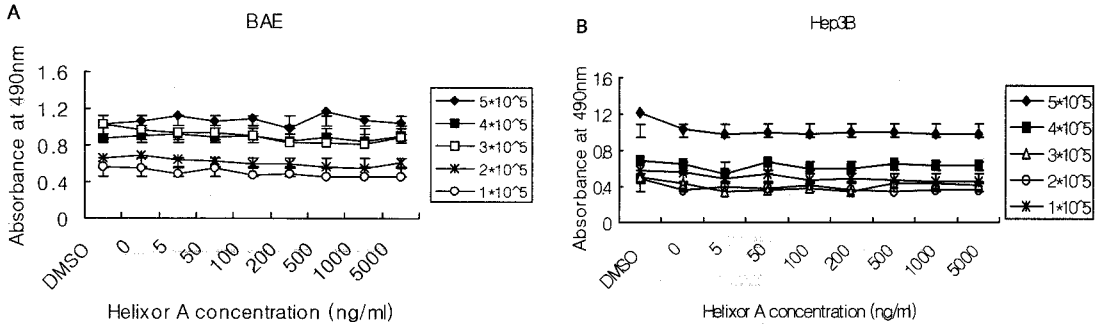

Fig. 1. The effects of Helixor A on cell cytotoxicity in BAE and Hep3B cells. Effect of Helixor A on cell viability was examined by MTT assays for BAE (A) and Hep3B (B) cells seeded at the density indicated. After cultured for 18h, cells were serum-starved for 24 h and treated with various concentrations of Helixor A for an additional 24 h prior to performing MTT assay. DMSO was also used to evaluate the effect of solvent for dissolving Helixor A. Data were expressed as mean ±S.D. of three independent experiments.

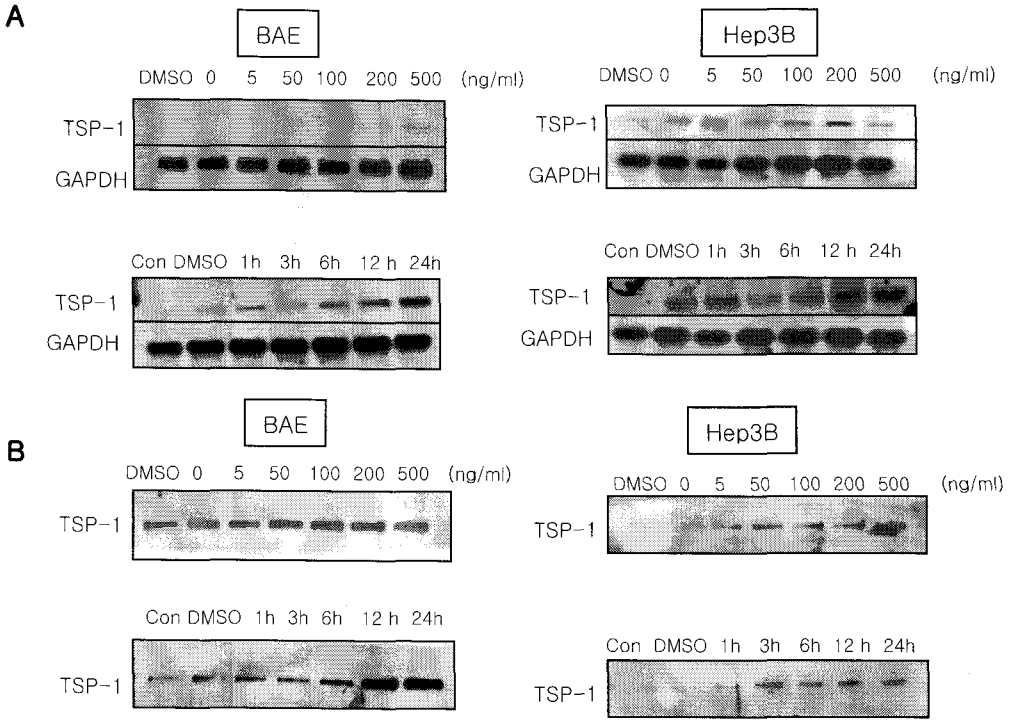

Fig. 2. Helixor A-induced upregulation of TSP-1 expression in a dose-dependent and a time-dependent manner in BAE and Hep3B cells. Cells were serum-starved for 24 h, and then treated with the indicated concentrations of Helixor A(200ng/ml) or DMSO for 24 h. (A) Northern blot analysis. Total RNAs were extracted form cells at the indicated time periods, electrophoresed on 1% agarose gel, capillary transferred to nylon membrane, and subjected to Northern blotting. The blot was hybridized with Digoxigenin-labeled TSP-1 cDNA probe. The same blot was stripped and reprobed with Digoxigenin-labeled GAPDH cDNA probe. (B) Western blot analysis. Proteins (20 μg/lane) from Whole cell conditioned media were separated on 8% SDS-polyacrylamide gel and analyzed by Western blot with mouse anti-TSP-1.

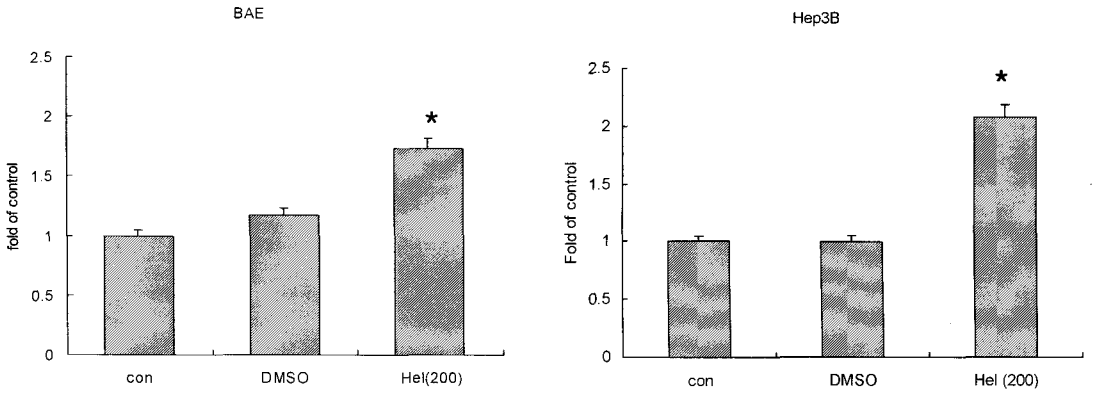

Fig. 3. Effect of Helixor A on activity of human TSP-1 promoter. Transient transfection studies of TSP-1 luciferase promoter construct in (A) BAE cells and (B) Hep3B cells, respectively. After twenty four hours following lipofectamin-mediated transfection of reporter plasmid containing luciferase gene with TSP-1 promoter (1 μg) into cells, the cells were left untreated or treated with Helixor A or DMSO for an additional 18 h prior to determining luciferase activity. Cotransfection of pCMV-β-gal (0.1 μg) plasmid was used to control for transfection efficiency. The data represent the mean±S.D. of the four independent experiments in promoter assay. *P<0.05 was doubly checked as Helixor A-treated group versus untreated or DMSO-treated group.

the invasion of cells by 40 % compared to the untreated conditioned media. Next, to determine whether the secreted TSP-1 from the Helixor-A treated conditioned media exerted negative effect on the invasion of cells, we neutralized the secreted TSP-1 with anti-TSP-1 antibodies and examined the effect of the conditioned media devoid of anti-angiogenic activity of TSP-1. As shown in Fig. 4, the inhibitory effect of Helixor A on chemotaxicity of BAE cells

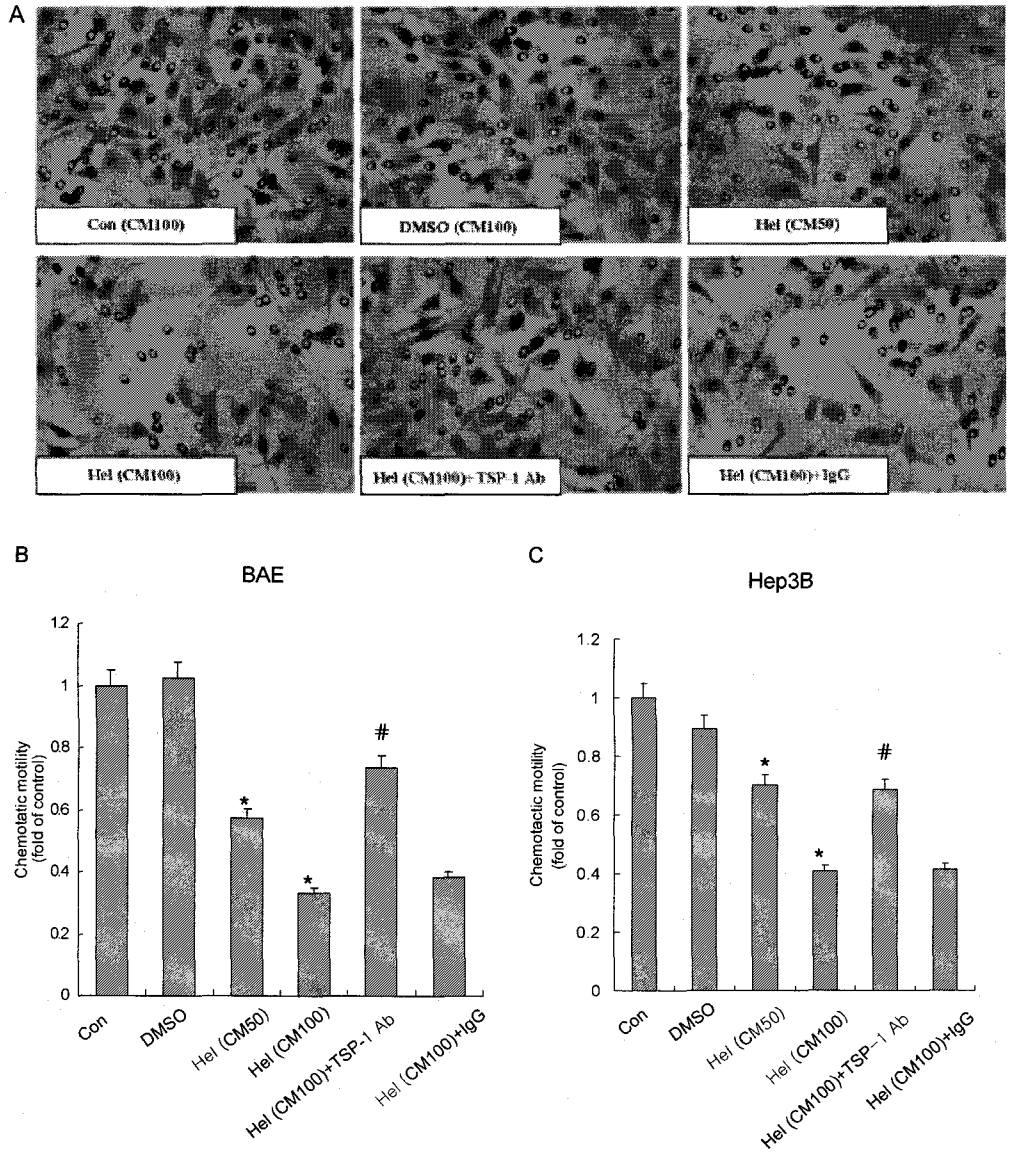

Fig. 4. Inhibition of cell invasion by Helixor A-induced TSP-1. A modified boyden chamber assay was used to examine the effect of Helixor A on cell invasion. Suspended cells were untreated or treated with Helixor A or DMSO CM, placed in the upper chamber, and allowed to invasive for 8~10 h to lower chamber containing 10% FBS as invasion inducer. For neutralization of TSP-1, cells were incubated in upper chamber following a combined treatment with Helixor A CM and neutralizing anti-TSP-1 antibodies (10 μg/ml) (A). BAE (B) and Hep3B (C). Cell invasion was expressed as relative fold to that of untreated control group. (A) The representative photographs of BAE cells taken at × 40. CM, conditioned media. *P<0.05 was determined as Helixor A-treated group versus con or DMSO group. #P<0.05 was determined as neutralizing TSP-1 antibody-trented roup versus Hel(CM100) group.

were also significantly blocked by TSP-1 neutralizing antibodies. Hence, TSP-1 might be a major mediator in Helixor A-stimulated BAE cell and Hep3B cell invasion.

Helixor A-upregulated TSP-1 inhibits capillary-like tube formation in BAE cell

The capillary-like tube formation has been considered as an important phenomenon of angiogenesis *in vitro* and *in vivo*. By using the Matrigel tube formation assay, we examined the effect of Helixor A-induced TSP-1 on angiogenic abilities of BAE cells (Fig 5). BAE cells were seeded on the surface of Matrigel, and then incubated in the presence of 100 μℓ CM of Helixor A-pretreated BAE cells for 24 h. The morphological changes were photographed at the indicated time. As shown in Fig. 5, Hel (CM50) and Hel (CM100) group, respectively, the cells treated with 50 or 100 μl CM

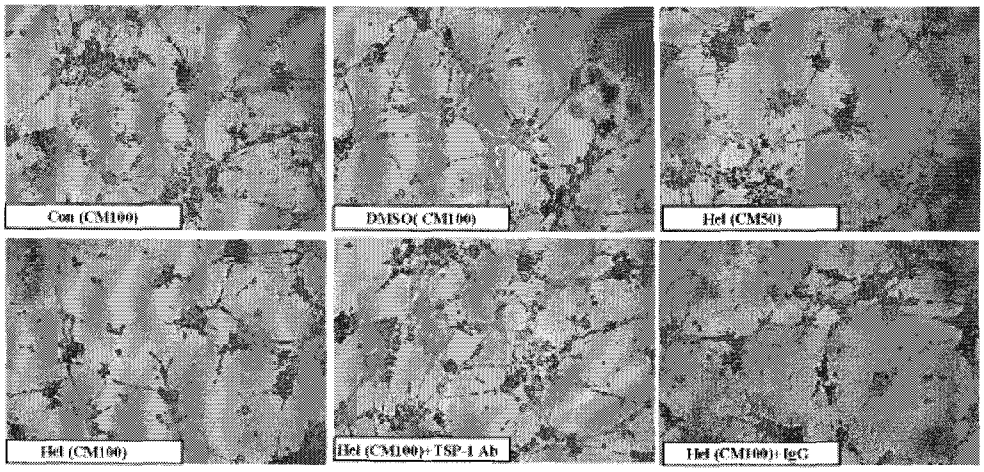

Fig. 5. Helixor A-upregulated TSP-1 inhibits capillary-like tube formation in BAE cells. BAE cells were plated on Matrigel-coated 48 well plate at a density of 1×10^5 cells/ml with or without Helixor A (200 ng/ml) pretreated CM. For neutralization of TSP-1 action, Helixor A-pretreated CM and 10 μg/ml TSP-1 neutralizing antibodies were added to cells. Cells were incubated for 12 h and morphological changes were determined. Morphology of cells was photographed in a inverted microscope (× 40).

significantly inhibited formation of tube-like structures, which was assessed by the reduced net-like structures compared to untreated control group. These results indicate that Helixor A plays an important role in anti-angiogenic process.

To further investigate the specific role of the induced TSP-1 in Helixor A-induced antiangiogenesis. We used neutralizing antibodies against TSP-1 to test whether TSP-1 was directly involved in the antiangiogenesis Helixor A provoked. While nonspecific antibody (IgG) did not influence the inhibitory of the conditioned media on tube formation, TSP-1 neutralizing antibodies remarkably recovered the Helixor A-induced inhibitory effect on angiogenesis, as was evident in that the morphology of net-like structure from group treated with anti-TSP-1 was comparable to that from the untreated control (Fig. 5, Con (CM 100) versus Hel (100)+TSP-1 Ab group). These results suggested that TSP-1 protein induced by Helixor A treatment plays as an important mediator in capillary-like tube formation.

Discussion

Angiogenesis, the formation of new blood vessels, is required for many pathologic processes, including invasive tumor growth as well as physiologic organ/tissue maintenance. It is evolved through a complex multifactor process that involves interaction of pro-angiogenic and anti-angiogenic signals from tumor, endothelial and stromal cells. Among several molecules controlling angiogenesis, Vascular endothelial growth factor (VEGF) and TSP-1 appear to be most relevant. Acummulating evidence indicate that VEGF is a key activator of angiogenesis [5] and TSP-1 is a primary endogenous inhibitor of angiogenesis[14].

Helixor A, aqueous mistletoe extracts, are widely used in complementary cancer therapy in addition to major cancer therapy. A number of components with different possible effects were isolated: lectins, viscotoxins, alkaloids, etc. According to numerous preclinical and *in vitro* studies the mistletoe extracts have immunomodulatory[9,18], cytotoxic, apoptotic and anti-angiogenic effects[16]. So far, specific effects of Helixor A on the immune system and its actual bearing on the tumour process have not been unequivocally elucidated.

In this study, we first tested the effect of Helixor A on TSP-1 expression in BAE cells and Hep3B cells and evaluated a role of Helixor A-induced TSP-1 in the cell motility and angiogenesis. In both cells Helixor A upregulated TSP-1 expression in both mRNA and protein levels in a dose-and time-dependent manner (Fig. 2). In our dose-dependency assay, we found that the minimum dose for induction of TSP-1 mRNA was 50 ng/ml for BAE and 100 ng/ml for Hep3B cells, respectively, although the secreted TSP-1 protein levels were still maintained at a maximum in both cells at 500 ng/ml of Helixor A. This concentration (500 ng/ml) of Helixor A, at which concentration Helixor

A was maximally effective in BAE cells, did not show the stimulatory effect on mRNA level in Hep3B cells. These findings suggest that maximum or optimal concentration for induction of TSP-1 is different among cell types [4]. This study also observed that the continuous increase in mRNA level up to 24 h was well correlated with subsequent enhanced secretion of TSP-1 protein into culture medium in both cells. These results are consistent with previous reports that enhanced secretion of TSP-1 was concomitant with increased mRNA level[12]. Therefore, it is quite possible that Helixor A increases the secretion of TSP-1 protein by modulating TSP-1 gene expression.

Our promoter analysis demonstrated that TSP-1 was markedly induced at the transcriptional level (Fig 3). The promoter of the TSP-1 gene contains multiple putative cis-elements for interaction with trans-acting factors in response to different extracellular stimuli. Since Helixor A contains several components such as lectins, viscotoxins and alkaloids, it is possible that various portions on the promoter were involved in the regulation of promoter activity. Thus, it is essential to determine the effect of each single component on the TSP-1 expression in the future study.

Helixor A-induced TSP-1 suppressed the invasive migration of Hep3B and BAE cells and capillary like tube formation of endothelial cells. In addition, the Matrigel system is one of the reliable models to assess angiogenesis in vitro. We revealed that the conditioned media treated BAE cells lead to an inhibition in chemotatic motility and the capillary-like tube formation (Fig 4 and 5). Moreover, our data were further supported by using TSP-1 neutralizing antibodies that TSP-1 antibody recovered Helixor A-induced antiangiogenesis. Since Helixor A is a complex containing various components, its physiological function is a combined effect resulting from competing between negative and positive factors in the Helixor A. Interesting, the final effect induced by Helixor A was disposed toward antiangiogenesis. Therefore, the antiangiogenic activity can be maximized by the isolated single component having antiangiogenic property.

In summary, our findings represents that Helixor A induces the TSP-1 expression in TSP-1 mRNA and protein levels. The induction by helixor A occurs at the transcription level. The upregulation of TSP-1 expression by Helixor A inhibits cell invasion and capillary-like tube formation, which is reversed by blocking TSP-1 activity with TSP-1 neutralizing antibodies Hence, Helixor A-induced antiangiogenic effect is caused by specific activity of TSP-1. Thus, our results suggest that Helixor A induced antiangiogenic effects be mediated by increasing of TSP-1, an antiangiogenic factor.

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초록 : Helixor A는 시험관 내에서 thrombospondin-1의 상승조절을 통해 신혈관생성을 억제한다.

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Thrombospondin-1은 암성장과 신혈관생성 억제조절인자로 병리학적 조건과 세포외에서의 자극에 의해 세포 특이적으로 조절되어지고 이 TSP-1 유전자 발현조절은 암치료제 개발을 위한 새로운 접근으로 중요하다. Mistletoe는 기생식물로 면역조절과 항암제로 사용되어지고 있다. Helixor A는 mistletoe 추출물의 수용액부분이다. 여기서 우리는 Helixor A를 투여하면 간암세포주 (Hep3B)와 혈관내피세포주 (BAE)에서 TSP-1의 mRNA와 단백질 발현이 유도되는 것을 관찰하였다. TSP-1 promoter 활성분석으로 TSP-1유전자의 발현이 Helixor A에 의해 전사단계에서 조절되어진다는 것을 확인하였다. 세포 침윤 분석에서 Helixor A를 처리한 배지를 두 세포주에 처리함으로 침윤된 세포의 수가 현저하게 줄어드는 것을 관찰하였고, Matrigel에서 혈관 내피 세포 (BAE)의 모세관 형성을 억제하는 것을 관찰하였다. 또한 세포 침윤과 모세관 형성에서 Helixor A를 처리했던 배지의 억제 효과가 TSP-1 중화 항체에 의해 그 효과가 상실되었다. 그러므로, 이 결과는 TSP-1이 Helixor A에 의해 유도되어진 혈관신생 억제 효과에 연관이 있음을 제시하였다. 이러한 결과를 종합 하였을 때 Helixor A가 TSP-1의 상승조절을 통해 혈관신생 억제 효과를 가질 것 이라고 생각된다.