Carbohydrate Polymers 88 (2012) 1326–1333

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

A polysaccharide from *Agaricus blazei* attenuates tumor cell adhesion via inhibiting E-selectin expression

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ARTICLE INFO

Article history:
Received 10 December 2011
Received in revised form 18 January 2012
Accepted 7 February 2012
Available online 16 February 2012

Keywords:
Polysaccharide
Agaricus blazei
Adhesion
E-selectin
NF-κB

ABSTRACT

Agaricus blazei Murill is an edible and medicinal mushroom of Brazilian origin, the components from which have been shown to possess anti-tumor potentials. Meanwhile, E-selectin-mediated cell–cell adhesion plays an important role in extravasation of tumor cells. In this study, the effect of a low molecular weight polysaccharide isolated from *A. blazei* (LMW-ABP) on the TNF-α-stimulated E-selectin protein and mRNA expression was evaluated in vitro. LMW-ABP could depress the E-selectin protein expression, and similar results were observed for E-selectin gene expression in a dose-dependent manner, which lead to a significant reduction in TNF-α stimulated adhesion of HT-29 cells to HUVEC. These results indicated that the effect of LMW-ABP against tumor cell adhesion was based on the inhibition of E-selectin protein and gene expression. In addition, LMW-ABP also suppressed nuclear factor-kappa B (NF-κB) protein expression and nuclear translocation. The data supported the view that LMW-ABP might be developed to a promising therapeutic agent against E-selectin-mediated neoplasm metastasis.

1. Introduction

Agaricus blazei Murill is a mushroom native to Brazil, and is widely used among cancer patients as complementary and alternative medicine (Ziliotto, Pinheiro, Barbisan, & Rodrigues, 2009). Some polysaccharides and protein-bound polysaccharides isolated from *A. blazei* have been shown to have anticancer activity directly or through immunomodulation (Itoh, Ito, Amano, & Noda, 1994). Polysaccharides have emerged as an important class of bioactive natural products and have many pharmacological functions, such as anticancer, anti-inflammatory, and immunopotentiating effects (Gamal-Eldeen, Ahmed, & Abo-Zeid, 2009). In recent years, many effective anticancer drugs have been developed (Fernández et al., 1992; Sze et al., 2006), and natural plant ingredients with anticancer effects have been attracted much attention by many pharmacologists (Salminen, Lehtonen, Suuronen, Kaarniranta, & Huuskonen, 2008).

The formation of hematogenous metastasis is a complex process by which tumor cells spread out from the primary tumor (Sehgal et al., 2005). A growing body of evidence indicates that the attachment of circulating tumor cells to the vascular endothelium of the target organ is thought to be one key step in the formation of hematogenous metastasis (Sugino et al., 2002, 2007). The binding of tumor cells to specific adhesion molecules of E-selectin on the surface of endothelial cells may mediate this process (Sahni, Arévalo, Sahni, & Simpson-Haidaris, 2009). Beside its well-known contribution to inflammatory responses, E-selectin also effectively works in the process of metastasis by promoting the adhesion of circulating tumor cells to the endothelium, which is a prerequisite for tumor cell extravasation (Wei et al., 2010). The correlation of the expression of E-selectin and tumor-specific expressed ligands for E-selectin in colon carcinoma cells with metastatic potential has been most convincingly demonstrated (Khatib, Fallavollita, Wancewicz, Monia, & Brodt, 2002). Therefore we can draw a conclusion that expression of E-selectin can be considered as one important factor promoting metastasis.

It is well established that the expression of various cell adhesion molecules is regulated by the transcription factor κB, a transcriptional activator of E-selection gene expression (Rajan, Ye, Bai, Huang, & Guo, 2008). The promoter regions of the genes for E-selectin contain nuclear factor-kappa B (NF-κB) binding site (Bucki et al., 2008). NF-κB is located in the cytoplasm in an inactive form associated with its inhibitors, the IκB proteins (Tang et al., 2010). In response to TNF-α stimulation, IκBs are phosphorylated by IκB kinase, which allows NF-κB to translocate to the nucleus (Kumar et al., 2007). As a result, agents that block NF-κB signaling also exert potential anti-metastasis effects (Li, Li, Tsao, & Cheung, 2009).

In our previous report, we have reported that LMW-ABP could suppress tumor growth and angiogenesis in vivo through down-regulation of vascular endothelial growth factor (VEGF) (Niu, Liu, Zhao, & Cao, 2009). In 2010, we evaluated the effect of LMW-ABP on the interaction between E-selectin and sialyl Lewis X (sLe^x) by flow

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doi:10.1016/j.carbpol.2012.02.015

cytometry and real time-reverse transcription polymerase chain reaction (RT-PCR) technology. The results suggested that LMW-ABP could inhibit sialyl Lewis X/E-selectin-mediated metastatic potential in HT-29 cells through down-regulating the expression of both α-1-3-fucosyltransferase-VII (FucT-VII) and sLe^x in transcription or translation level, respectively (Liu et al., 2010). It is uncertain whether LMW-ABP also has similar suppressing effect on the expression of E-selectin protein or mRNA. Therefore, in present study we intend to investigate the role of TNF-α-induced E-selectin expression influenced by LMW-ABP in the adhesion of HT-29 cell to HUVECs. At the same time we also examined potential underlying mechanisms via the NF-κB signaling pathway.

2. Materials and methods

2.1. Materials

A. blazei was purchased from Zhejiang Bo Rui Pharmaceutical Co. (Qingyuan, China). HT-29 human colon cancer cells and HUVECs were purchased from ATCC (Manassas, VA, USA). Mouse anti-human E-selectin monoclonal antibody and FITC-conjugated anti-human IgG were obtained from BD Bioscience (Bedford, MA, USA). Mouse anti-NF-κB (p65) monoclonal antibody and anti-goat IgG-HRP were purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). TNF-α was obtained from R&D Systems Inc. (Minneapolis, MN, USA). Medium RPMI 1640, fetal calf serum (FCS) and trypsin were from GIBCO BRL (Grand Island, NY, USA). Cell culture growth media (McCoy's 5A, F-12K) and PDTC were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RT-PCR kit was purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). The primers were synthesized by Shanghai Sheng-Gong Biotechnology Co. (Shanghai, China). The enhanced chemiluminescence (ECL) detection kit was purchased from GE Healthcare (Waukesha, WI, USA). Calcein-AM and Trizol were purchased from Invitrogen Co. (Carlsbad, CA, USA). All other reagents were from various china chemical reagent manufacturers and chemical reagent suppliers, and were of analytical grade unless otherwise stated.

2.2. Extraction and purification of LMW-ABP

LMW-ABP was extracted from *A. blazei* with a method as previously described (Liu et al., 2010). Briefly, after the fruiting bodies of *A. blazei* were defatted with 80% EtOH under reflux. The residue was then extracted with distilled water at 75 °C for 3 times and 2 h each time. Subsequently, the whole combined extract was filtered, concentrated and centrifuged, and then the supernatant was precipitated with 95% EtOH (1:5, v/v) at 4 °C for 12 h. The precipitate collected by centrifugation was deproteinated by a combination of proteinase and Sevag method (Staub, 1965) and exhaustively dialyzed against water for 48 h. The dialysate was precipitated by adding 5 vol. of 95% ethanol to obtain the crude polysaccharide, named as CABP.

The CABP was further purified with DEAE-650M column, eluted successively with different concentrations of NaCl aqueous solution (0 and 1 M) stepwise at 8 mL/12 min. Fraction was collected and monitored with the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). One main fraction eluted with 0.1 M NaCl was collected, dialyzed, lyophilized, and was further purified on Toyopearl HW-65F and Toyopearl HW-50S columns in turn with 0.2 M NaCl with a flow rate of 0.5 mL/min. The eluted fractions containing a large amount of sugar were only separated into one fraction and precipitated by 5 vol. of 95% ethanol to obtain purified polysaccharide, named as LMW-ABP. A stock LMW-ABP solution was prepared by dissolving in PBS (vehicle) and sterilized by passing it through a 0.22 μm millipore filter for further analysis.

2.3. Cell culture

The HT-29 human colon cancer cell line were cultured in McCoy's 5A medium supplemented with 10% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 4 mM glutamine at 37 °C in a humidified atmosphere of 5% CO_2. HUVECs were cultured in F-12K medium supplemented with 0.1 mg/mL heparin, 0.04 mg/mL endothelial cell growth supplement (Sigma), and 10% FCS at 37 °C.

2.4. Cell adhesion experiments under static conditions

A monolayer cell adhesion assay was performed as described previously (Schuldes et al., 2003) with slight modification. Briefly, HUVECs in exponential phase of growth were trypsinized and inoculated to each 96-well microtiter plate (3×10^4 cells/well). HUVECs were pretreated with varying concentrations of LMW-ABP (5, 10, and 20 μg/mL) for 48 h. In the ligand antagonist assay, HUVECs were incubated with a neutralizing antibody for E-selectin at room temperature for 30 min. HT-29 cells were washed with RPMI1640 medium and labeled with 5 μM of Calcein AM. After 20 min of incubation at room temperature in dark, the tumor cell was washed with PBS. Subsequently, HUVECs were incubated with 5 ng/mL TNF-α at 37 °C for 4 h before the cell adhesion experiment of HT-29. After the washing of HUVECs with RPMI1640 medium twice, HT-29 cells (200 μL, 5×10^4 cells/well) were added to HUVECs and incubated at room temperature with rotation (120 rpm). Thereafter, HUVECs were washed gently twice with PBS containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ to remove residual non-adherent tumor cells. The fluorescence intensity at 520 nm was measured on an ELISA reader (Safire 2, TECAN). Each sample had three dishes, and each dish was measured for 3 min. The mean value was calculated from five trials. The HUVECs was treated by vehicle was the control. The percentage of adhesion was calculated on the assumption that 200 μL of Calcein-AM labeled cell suspension represents 100%. The rate of adhesion and inhibition were calculated according to the formula below:

$$\text{Adhesion rate}\,(\%) = \frac{\text{the mean fluorescence value of samples}}{\text{the mean fluorescence value of control group}} \times 100$$

$$\text{Inhibiting rate}\quad (\%) = (1 - \text{tumor cell adhesion rate}) \times 100$$

2.5. Analysis of E-selectin mRNA expression by RT-PCR

The expression of the mRNA transcript of E-selectin was evaluated by RT-PCR as described previously (Alvarez et al., 2010). In brief, HUVECs were pretreated by different LMW-ABP samples (5, 10, and 20 μg/mL) for 48 h and then incubated with 5 ng/mL TNF-α at 37 °C for 4 h. Total RNA was isolated using Trizol Reagent. The reverse transcription (RT) reaction with 2 μg of total RNA was performed using a RT-PCR Kit. The PCR reactions were carried out in a GeneAmps System 9700 (PE Biosystems, Foster City, CA). PCR reaction primer sets was as follows: forward primer, 5′-CCCTCCTGACAT TAGCAC-3′; reverse primer, 5′-CATAGTAACCCTCGCACA-3′ for E-selectin; forward primer, 5′-GTGGACATCCGCAAAGAC-3′; reverse primer, 5′-GAAAGGGTGTAACGCAACT-3′ for β-actin. The PCR cycles for each reaction were as follows: heat denaturation at 94 °C for 5 min and cycled 30 times at 94 °C for 30 s, 50.2 °C for 30 s and 72 °C for 1 min, and this was followed by an additional extension step at 72 °C for 5 min. PCR products were electrophoresed on 1% agarose gels and followed by ethidium bromide staining and photography. Band intensities were quantified using AlphaEaseFC® Imaging software (AlphaInnotech, San Leandro, CA).

2.6. Flow cytometry analysis

The expression of E-selectin was analyzed by flow cytometry. HUVECs (1×10^6 cells/well) in logarithmic phase adhered to bottom of 6-well culture plates, and then this system was incubated with different LMW-APB samples (5, 10, and 20 μg/mL) for 48 h and incubated with 5 ng/mL TNF-α at 37 °C for 4 h before the analysis. Thereafter HUVECs (1×10^6) were collected and washed twice in a cold solution of PBS containing 0.5% FCS. Cells (1×10^6) were then resuspended in 1% PBS-BSA solution containing appropriate concentrations of the monoclonal antibody against E-selectin (4 μL for 10^6 cells). 4 μL PBS were used as controls. After 30 min incubation at 4 °C, cells were washed twice, and were marked by 5 μL FITC-conjugated goat anti-mouse IgG antibody for 30 min at 4 °C in the dark. Then the cells were subjected to fluorescence analysis performed on a FACS Calibur flow-cytometric apparatus (Becton Dickinson, Franklin Lakes, NJ) after suitable washing. Data were expressed as the mean fluorescence intensity or the number of positive cells. Each experiment was performed in triplicate.

2.7. Western blot

HUVECs were lysed with a buffer containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitors. Then, lysates were centrifuged at 15,000 × *g* at 4 °C for 10 min. The total cell lysate was separated by SDS-PAGE (12% running, 6% stacking) after normalization for protein content and transferred onto a polyvinylidene difluoride membrane (0.45-μm pore size). The membranes were incubated overnight at 4 °C with the monoclonal antibody against NF-κB and monoclonal antibody against E-selectin in TBS, and were washed three times for 10 min in TBS before the addition of the secondary antibody (rabbit anti-mouse IgG HRP in a final dilution of 1:5 000) in TBS containing 0.1% nonfat dry milk for 2 h. The membranes were then washed in TBS for 10 min followed by water for 5 min. Reactive bands were identified using enhanced chemiluminescence and autoradiography according to the manufacturer's instructions.

2.8. Immunocytochemistry

The translocation of the NF-κB subunits p65 from the cytosol to the nucleus was assessed by immunohistochemistry. The cells were treated as described above for the adhesion assay. Confluent HUVECs monolayer on glass coverslips were fixed with 95% alcohol at 4 °C for 20 min and then incubated for 20 min in goat serum to block non-specific staining. NF-κB mouse monoclonal antibody (1:50) was dropwise add to the glass coverslips and then incubated at 4 °C overnight. Immune complexes were detected with the Vectastain ABC kit and DAB substrate for peroxidase (Vector, Burlingame, CA, USA). The cells were examined with an Olympus microscope.

2.9. Statistical analysis

The data are presented as means ± S.D. Each experiment was performed at least in duplicate and each assay at least in triplicate. Differences between the groups were assessed by analysis of variance (ANOVA). When the ANOVA test was significant, a Newman–Keul test was performed between the groups. All statistical analysis was performed using SPSS version 13.0 (SPSS Inc., Chicago, Illinois). *P*-values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. The effect of LMW-ABP on the TNF-α induced adhesion of human tumor cell to endothelial cells

To examine the effects of LMW-ABP on HT-29 tumor cell adhesion to HUVECs, a monolayer cell adhesion assay was carried out. As shown in Fig. 1, the adhesion of HT-29 cells to HUVECs was strongly induced on stimulation with TNF-α (5 ng/mL). This binding was significantly blocked by the addition of anti-E-selectin antibodies [Fig. 1A(f)], thus confirming that cellular binding mainly depends on protein–carbohydrate interactions. Pretreatment of HUVECs with varying concentrations LMW-ABP (5, 10, and 20 μg/mL) inhibited the adhesion with HT-29 cells in a dose-dependent manner.

3.2. The effect of LMW-ABP on the E-selectin expression induced by TNF-α in endothelial cells

As the expression of cell adhesion molecules on endothelial cells is a prerequisite for adhesion of tumor cells, the effect of LMW-ABP on TNF-α-induced expression of E-selectin was investigated. The results using flow cytometry demonstrated that E-selectin was expressed at low levels on unstimulated HUVECs and there was more than 10-fold increase in their expression upon stimulation with TNF-α. Pretreatment of HUVECs with varying concentrations LMW-ABP (5, 10, and 20 μg/mL) significantly inhibited TNF-α-induced E-selectin expression levels in a dose-dependent manner (Fig. 2). The mRNA levels of the E-selectin were measured by RT-PCR in order to further clarify the mechanism responsible for the changes in the level of E-selectin protein. Pretreatment of HUVECs with LMW-ABP markedly also decreased the E-selectin mRNA levels (Fig. 3). This suggested that LMW-ABP suppressed E-selectin expression at the transcriptional level, and lowered the production of their protein and reduced the level of HT-29 cells adhesion.

3.3. The effect of LMW-ABP on NF-κB

The promoter region of E-selectin gene contains NF-κB binding elements. To investigate whether the inhibitive effect of LMW-ABP on TNF-α induced E-selectin expression was relative to NF-κB in HUVECs, we also measured NF-κB p65 in nuclear extracts from untreated cells and cells treated with TNF-α in the presence or absence of LMW-ABP. NF-κB p65 markedly increased 2 h after stimulation with TNF-α from very low levels. This increase of NF-κB p65 was significantly blunted by 33% and 66%, respectively, in the presence of 10 μg/mL and 20 μg/mL of LMW-ABP (Fig. 4). We further determined the effect of LMW-ABP on the E-selectin levels. The E-selectin level in HUVECs was significantly inhibited by 41% when HUVECs were treated with LMW-ABP (20 μg/mL) compared with untreatment cells (Fig. 5). Similarly, PDTC, a potent inhibitor of phosphorylation of NF-κB, also decreased E-selectin level by 58% relative to unstimulated levels in TNF-α-treated cells.

In addition, the effect of LMW-ABP on TNF-α-induced nuclear translocation of NF-κB as assessed by indirect immunohistochemical microscopy was well in agreement with the results obtained by western blot (Fig. 6). NF-κB immunoreactivity was low in unstimulated cells, most intense in TNF-α-treated cells, and moderate in LMW-ABP (20 μg/mL) plus TNF-α-treated cells. Furthermore, pre-treating endothelial cells with LMW-ABP (20 μg/mL) for 48 h significantly inhibited the TNF-α-induced NF-κB-DNA binding activity in HUVECs. These results suggested that the inhibition effect of LMW-ABP to TNF-α induced E-selectin expression was regulated by NF-κB pathway.

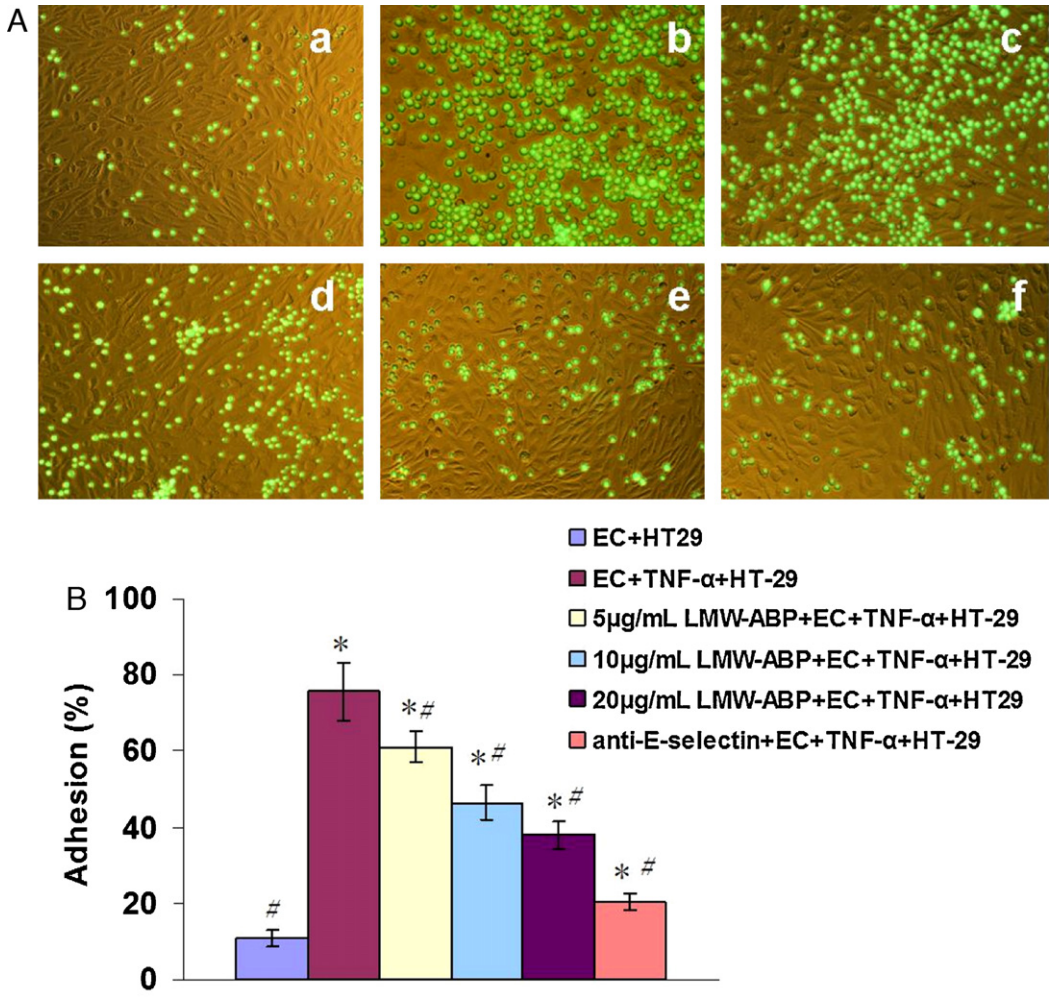

Fig. 1. The effect of LMW-ABP on the TNF-α-induced adhesion of HT-29 cells to endothelial cells.

4. Discussion

In our previous study, we reported that LMW-ABP significantly reduced the invasion of BEL-7402 hepatic cancer cells in vitro, and decreased lung metastatic foci in mice B16 melanoma models in vivo. In addition, LMW-ABP could inhibit the growth of not only the right, but also the left non-treated tumor in double grafted S180 mice tumor models (Niu et al., 2009). Although we had draw a conclusion that LMW-ABP could inhibit sialyl Lewis X/E-selectin-mediated metastatic potential in HT-29 cells through down-regulating the expression of both α-1-3-fucosyltransferase-VII (FucT-VII) and sLe^x in transcription or translation level, respectively (Liu et al., 2010). However the possible underlying anti-metastatic mechanisms of LMW-ABP remain to be not fully elucidated about the E-selectin expression on the HUVECs. Therefore, in the present study we investigated the effect of LMW-ABP on the expression of E-selectin and tumor cell adhesion to the endothelium.

Increasing evidence suggests that endothelial cell adhesion molecules play a role in tumor metastasis (Chirivi, Nicoletti, Remuzzi, & Giavazzi, 1994). Modulation of expression of the cell adhesion molecule E-selectin can be considered as a promising strategy for tumor therapy (Bhaskar et al., 2003). The eradication of metastatic disease is crucial for achieving survival in most patients with cancer (Hennessy et al., 2005). Metastasis is a complex process consisting of multiple steps, including invasion of extracellular matrix, extravasation into vessels, migration into the bloodstream, adhesion to endothelial cells in a new tissue, extravasation through the vessel wall, and migration and proliferation in response to organ-specific factors at the new site (Voura, Sandig, & Siu, 1998). The adhesion of circulating cancer cells to capillary endothelia is a critical step in the initiation of metastasis (Cheng, Abdel-Ghany, Elble, & Pauli, 1998). In the current study, we found LMW-ABP inhibited HT-29 cells adhesion to HUVECs stimulated by TNF-α. After pretreatment of HUVECs with LMW-ABP at concentration of 5, 10, and 20 μg/mL for 48 h following exposure to 5 ng/mL TNF-α for 4 h, the adhesion inhibitory rates were 15%, 21%, and 34%, respectively. The concentrations of TNF-α we used are comparable with other studies showing induction of E-selectin on HUVECs (Kang et al., 2006).

The specific interactions between selectins in endothelial cell of potential target organs and their counterpart ligands of sLe^a and sLe^x on tumor cell of different entities and their expression profiles appear to be important for the organ-specific distributions of metastases and correlate with the metastatic potential of several types of cancers (Hakomori, 1992). One of the first lines of evidences for a significant role of selectin-mediated adhesion mechanisms in the metastatic cascade was established when it was shown that the development of experimental liver metastases from human colorectal carcinoma cells was dependent on E-selectins (Brodt et al., 1997). E-selectin was considered to play a primary role in initiating the adhesion of cancer cells to vascular endothelial cells through its interaction with its specific ligand sialyl Lewis antigens (Schuldes et al., 2003). Interestingly, there seems to be

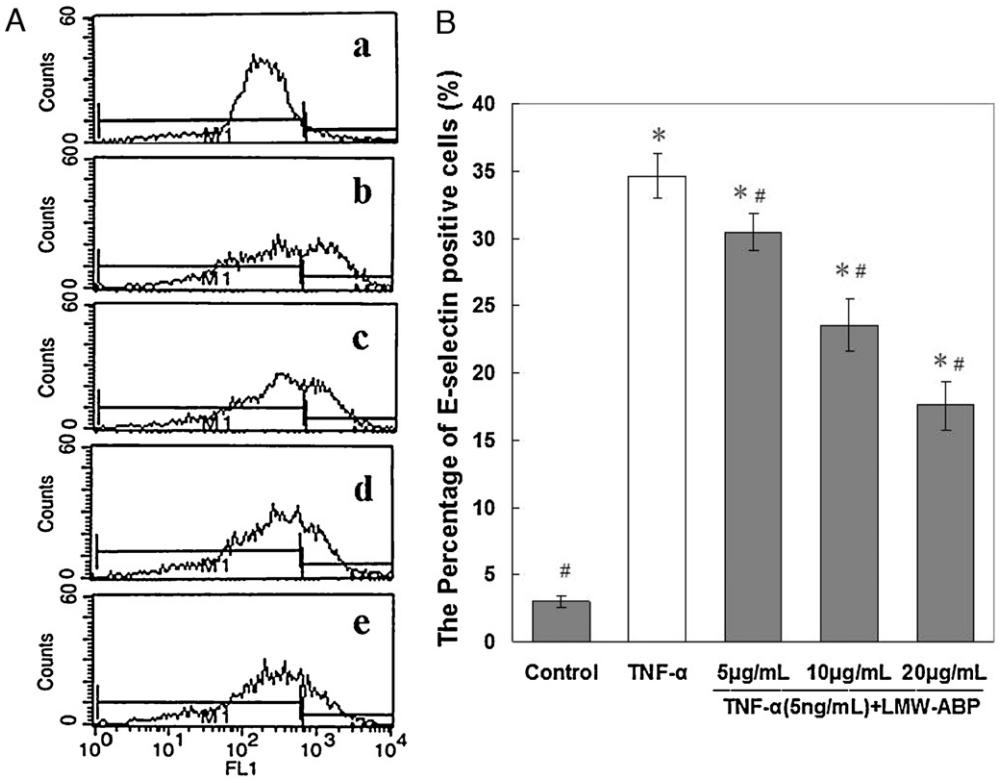

Fig. 2. The effect of LMW-ABP on the E-selectin of protein expression.

a bidirectional signaling between the tumor cell 'seeds' and the surrounding microenvironmental 'soil'. For example, endothelial cell adjacent to metastatic lesions expressed more E-selectin than endothelial cell adjacent to the primary tumor (Nakagawa et al., 2009). In addition, the rates of selectin expression were inversely correlated with the distance of blood vessels to the cancer cell nests (Ye et al., 1995). A vascular expression of E-sclectin was detected in 52% of the tumors compared with 21% in the normal breast tissues (Fox, Turner, Gatter, & Harris, 1995). Therefore,

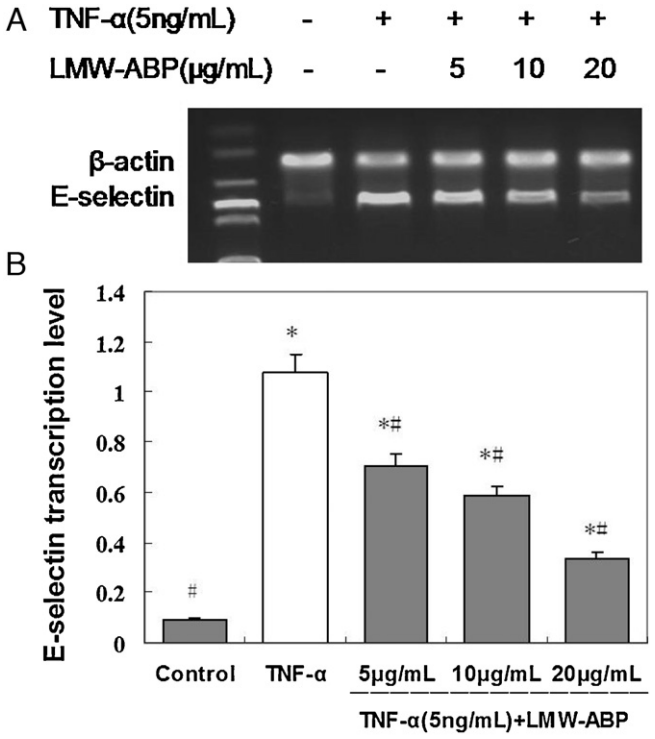

Fig. 3. The effect of LMW-ABP on the E-selectin of gene expression.

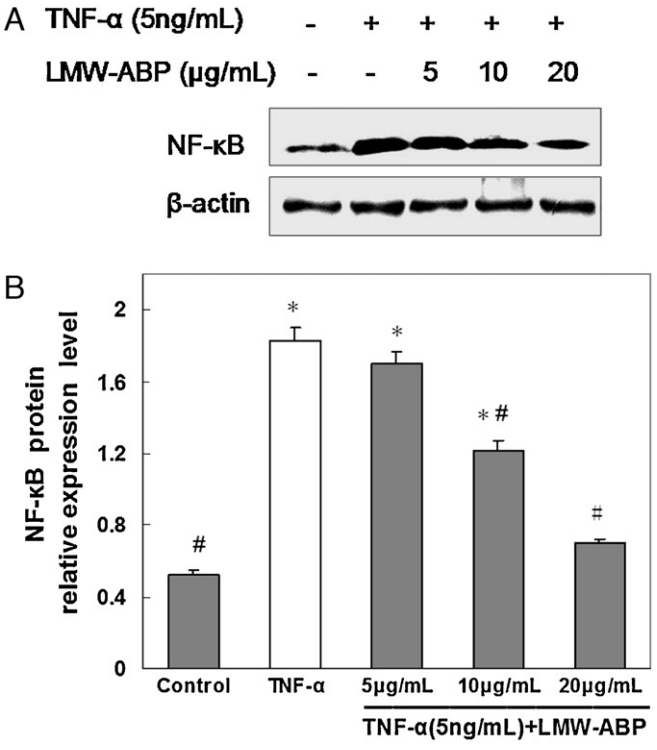

Fig. 4. LMW-ABP attenuates TNF-α-induced activation of transcription factor NF-κB p65 in HUVECs.

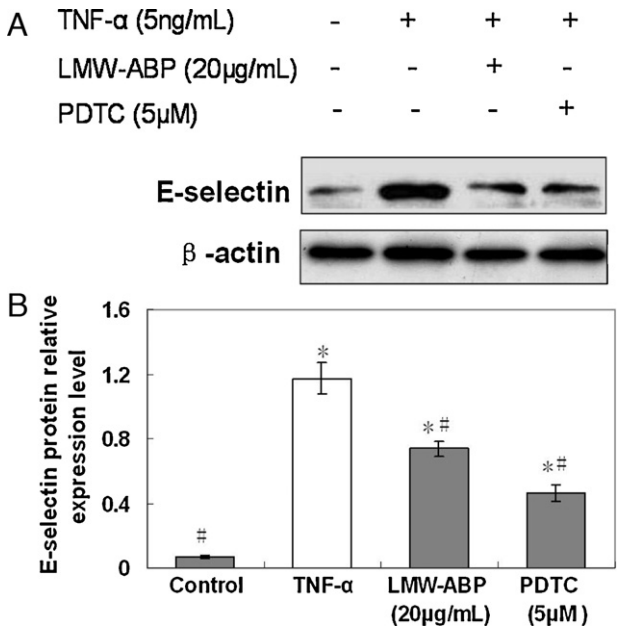

Fig. 5. Protein expression of E-selectin was analyzed by western blot analysis.

selectin expression appears to be inducible by some stimuli from cancer cells (Nooteboom, van der Linden, & Hendriks, 2004). Although the results of some studies concerning the involvement of these receptors remain controversial, there are several other carcinomas where the metastatic dissemination appears to be affected by selectin-mediated interactions. For example, MCF-7 and T-47D breast carcinoma cells accumulated and rolled under flow conditions on TNF-α-stimulated, but not on unstimulated endothelial cell monolayers (Rucci et al., 2004; Tözeren et al., 1995). These adhesive interactions were diminished or completely blocked by treatment of stimulated endothelial cell with an anti-E-selectin antibody (Fujii et al., 2000). Several lines of evidence indicated that TNF-α can up-regulate the endothelial expression of E-selectin, which mediated the interactions of tumor cells with the endothelium cells (Nawa et al., 2000). Moreover, metastatic spread to the liver of a Lewis lung carcinoma subclone was inhibited by an anti-E-selectin antibody (Khatib et al., 1999), implicating E-selectin as a crucial adhesion molecule in tumor metastasis. It has been suggested that adhesion molecules on cancer cells and vascular endothelial cells are closely related to the process of tumor metastasis. Human colon cancer cells adhere to HUVECs through the interaction of E-selectin on endothelial cells and sLe^x on cancer cells under static conditions (Yoshida et al., 1999). This study found that LMW-ABP inhibits TNF-α-induced E-selectin expression in HUVECs. The mRNA expression of E-selectin was also down-regulated by LMW-ABP treatment. In summary, we demonstrated here that LMW-ABP inhibited HT-29 cell adhesion to HUVECs stimulated by TNF-α by suppressing the gene expression of E-selectin.

The promoter regions of the genes encoding for E-selectin have binding sites for NF-κB (Schindler & Baichwal, 1994). As in other cell types, NF-κB is present in the cytoplasm of unstimulated endothelial cells, and it is translocated to the nucleus following stimulation by TNF-α (Castro-Alcaraz, Miskolci, Kalasapudi, Davidson, & Vancurova, 2002). Nuclear translocation of NF-κB induces the transcription of E-selectin genes (Yu, Rux, Ma, Bdeir, & Sachais, 2005). It is noteworthy that several therapeutic approaches to metastasis are based on the inhibition of the nuclear translocation of NF-κB, thus reducing the expression of E-selectin (Ichikawa et al., 2006). PDTC, a NF-κB inhibitor, completely inhibit NF-κB-dependent transcriptional activation after TNF-α stimulation. As we know rapidly migrating tumor cells may induce the expression of TNF-α, in turn TNF-α would soon enhance the expression of E-selectin. As a result, the first step of tumor colonization to an organ is enhanced, namely the interaction between tumor and endothelium cells. Our results also showed that the TNF-α stimulated endothelial cells had a higher NF-κB p65 expression. LMW-ABP distinctly inhibited NF-κB expression in HUVECs treated with TNF-α. In addition, LMW-ABP treatment markedly suppressed the TNF-α-induced NF-κB p65 nuclear translocation. The inhibitory effect of LMW-ABP on E-selectin expression was mediated by blocking NF-κB pathway in HUVECs. The results presented in this report provided the other corresponding insight into the mechanism responsible for the anti-metastasis activity of

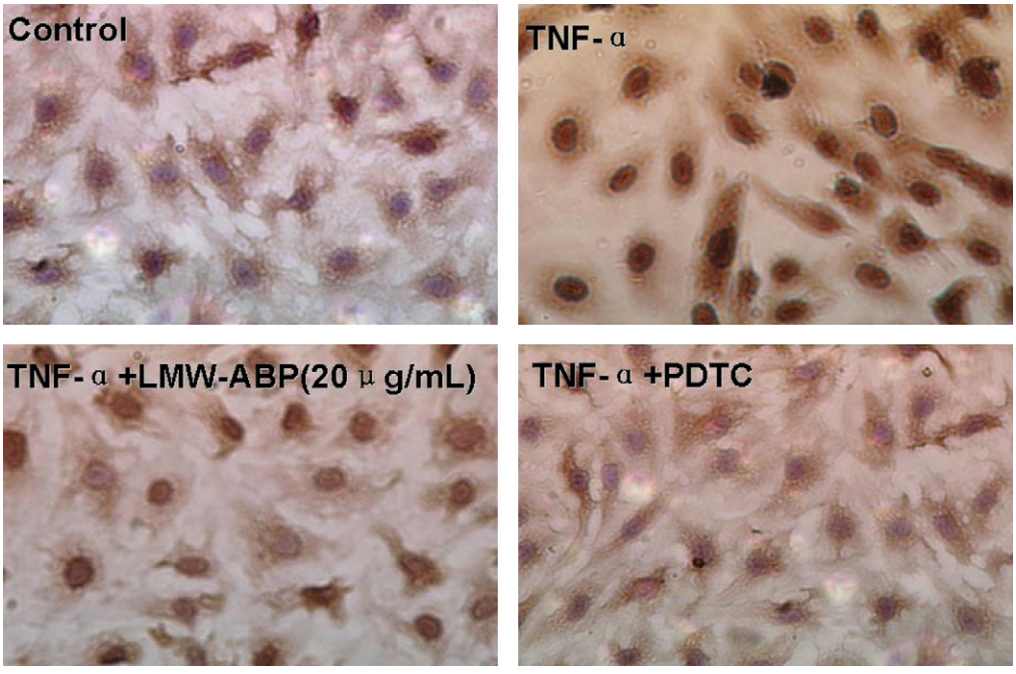

Fig. 6. Effect of LMW-ABP on TNF-α-induced nuclear translocation of NF-κB p65 in HUVECs.

LMW-ABP, mediated by E-selectin on HUVECs, not only sLe^x on tumor cell.

In summary, we can see that LMW-ABP attenuated TNF-α-stimulated increase of E-selectin expression in the HUVECs. The inhibitory effect of LMW-ABP on E-selectin expression was paralleled by disrupting the adherence of HT-29 cells and HUVECs. Furthermore, we also confirmed that TNF-α-induced NF-κB up-regulation was attenuated by LMW-ABP, and LMW-ABP inhibited NF-κB p65 translocation from cytosol to nucleus. From all the above demonstrations, we can make the following conclusions that: (1) LMW-ABP blocked the early metastasis process involving endothelial expression of inducible E-selectin; (2) LMW-ABP blocked not only HT-29 cells adhesion on the TNF-α-activated endothelium but also the activation of E-selectin expression. (3) The inhibition of E-selectin expression by LMW-ABP was at least in part mediated via the affecting NF-κB signaling pathway. This effect of LMW-ABP might have implications for strategies preventing and attenuating hematogenous metastasis.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 30772751) and the Special Foundation for Young Scientists of Heilongjiang province, China (No. QC2008C28).

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