Effects and mechanisms of curcumin and basil polysaccharide on the invasion of SKOV3 cells and dendritic cells

JING LV[1*], QIANQIAN SHAO[1*], HUAYANG WANG[1], HUAN SHI[1], TING WANG[1,2], WENJUAN GAO[1], BINGFENG SONG[1], GUANGJUAN ZHENG[2], BEIHUA KONG[3] and XUN QU[1]

[1]Institute of Basic Medical Sciences, Qilu Hospital of Shandong University, Jinan, Shandong 250012; [2]Department of Pathology, Shandong University of Traditional Chinese Medicine, Changqing University Science and Technology Park, Jinan, Shandong 250355; [3]Department of Gynaecology and Obstetrics, Qilu Hospital of Shandong University, Jinan, Shandong 250012, P.R. China

Received March 17, 2013; Accepted September 2, 2013

DOI: 10.3892/mmr.2013.1695

Abstract. In the present study, a polysaccharide extract was obtained from *Ocimum basilicum* (basil polysaccharide, BPS) and the effects of curcumin and BPS on the invasion activity of the SKOV3 ovarian cancer cells and human monocyte-derived dendritic cells (DCs) were investigated. SKOV3 cells and immature or mature DCs were treated with 50 μM curcumin or 100 μg/ml BPS. A transwell invasion assay demonstrated that curcumin and BPS differentially regulate the invasion of SKOV3 cells and DCs. Curcumin significantly decreased the invasion of SKOV3 cells and immature and mature DCs, while BPS only decreased SKOV3 cell invasion. Osteopontin (OPN) mRNA and protein expression were significantly reduced in curcumin and BPS-treated SKOV3 cells and curcumin-treated DCs. Furthermore, flow cytometry showed that curcumin significantly inhibited the surface expression of CD44 in SKOV3 cells and DCs, while BPS had a minimal effect on CD44 expression. Matrix metallopeptidase-9 (MMP-9) mRNA and protein expression were also reduced in all curcumin-treated cells and BPS-treated SKOV3 cells. The results indicated that curcumin and BPS regulated invasion of SKOV3 cells and DCs by distinctly downregulating OPN, CD44 and MMP-9 expression. Therefore, Curcumin and BPS may be suitable candidates for DC-based vaccines for ovarian cancer immunotherapy.

Correspondence to: Dr Xun Qu, Institute of Basic Medical Sciences, Qilu Hospital of Shandong University, 107 Wenhuaxi Road, Jinan, Shandong 250012, P.R. China
E-mail: quxun@qiluhospital.com

*Contributed equally

Key words: curcumin, *Ocimum basilicum*, ovarian cancer, dendritic cells, cell invasion

Introduction

Ovarian cancer is one of the most common types of malignancy in females worldwide, with an estimated 225,500 novel cases and 140,200 cancer-related mortalities occurring annually (1). Although chemotherapy for ovarian cancer has been well established throughout the last three decades, the 5-year survival rate remains <50%, the lowest of all gynecological malignancies. The cause for this is that ovarian cancer tends to have distantly metastasized by the time the majority of cases are diagnosed (1). Local invasion is an initiating step in cell migration and commonly occurs prior to distant metastasis; therefore, decreasing invasion activity of cancer cells may improve the outcome of ovarian cancer patients.

In healthy individuals, malignant cells are under the surveillance of immune cells to resist invasion and metastasis, which is an essential aspect of anticancer immunity. Dendritic cells (DCs) are known to be the most specialized antigen-presenting cells and are important in the development of anticancer immune responses as they control the initiation and polarization of adaptive immunity (2). A previous clinical trial showed promising results achieved by DC vaccination in advanced ovarian cancer patients, with 90% overall survival during 36 months of observation (3). Fulfilling this function, however, is dependent upon the migration of DCs to T-cell-rich lymph organs following the uptake of the vaccination and the presentation of tumor antigens. Inhibition of DC invasion activity facilitates tumor escape from host immune surveillance. A previous study indicated that intratumoral injection of DCs generated *in vitro,* fails to initiate systemic antitumor immunity as the DCs migrated into regional lymph nodes less efficiently (4). Thus, a complementary therapy, which decreases the motility of cancer cells without markedly affecting DC invasion, may be beneficial in preventing ovarian cancer metastasis.

Cell invasion results from cross-talk between cells and the extracellular matrix (ECM). Osteopontin (OPN), a phosphorylated glycoprotein with pleiotropic properties, is expressed on the ECM and on the surface of a number of cells, including malignant cells, lymphocytes and vascular smooth muscle cells. OPN promotes cell adhesion and invasion in various

physiological or pathological conditions (5-7). These functions are primarily achieved by its interaction with receptors on the cell surface, including the α5β3 integrin and CD44 (8), and the initiation of downstream signaling events, for example, matrix metallopeptidase-9 (MMP-9) expression (9-11). A previous study suggested that OPN decisively enhanced the migration of DCs into draining lymph nodes (12). In ovarian cancer, OPN serves as one of the biomarkers for early detection and predictors of prognosis (13,14), indicating its involvement in cancer development and progression. However, the specific mechanisms of OPN in ovarian cancer invasion remain unknown.

Among chemotherapeutic agents against cancer, natural bioactive molecules possess a unique advantage of milder side-effects. Curcumin, a polyphenolic pigment extracted from turmeric (*Curcuma longa*), is a prime example, due to its low toxicity and high anticancer potency. The application of curcumin as a complementary therapy for ovarian cancer appears promising as it induces apoptosis (15) and sensitivity to cisplatin (16) in ovarian carcinoma, without decreasing quality of life (17). Moreover, the enhancement of adaptive immunity was involved in curcumin-mediated tumor growth retardation (18,19). However, to the best of our knowledge, no systematic analysis of curcumin on the invasion of ovarian cancer cells and DCs has been reported. Sweet basil (*Ocimum basilicum*) is commonly used in Chinese traditional medicine for detumescence, anti-inflammation and promoting circulation, and previous studies have shown its cytotoxic effects in cancer cells (20). In the current study, a polysaccharide extraction was obtained from *Ocimum basilicum* (basil polysaccharide, BPS) and was compared with curcumin for its ability to effect the regulation of invasion of SKOV3 ovarian cancer cells and DCs. The underlying mechanisms were investigated. The results indicated that curcumin and BPS significantly inhibit the invasion of SKOV3 cells, while curcumin prevented the invasion of DCs to a greater extent compared with BPS. This diversity was achieved, at least partly, by distinctly regulating OPN, CD44 and matrix metallopeptidase-9 (MMP-9) expression.

Materials and methods

Materials. For flow cytometry, the following monoclonal antibodies: anti-CD14, anti-CD1a, anti-CD80, anti-CD86, anti-CD83, anti-human leukocyte antigen (HLA)-DR, anti-CD209, anti-CD44 and their isotype antibodies were purchased from BD-Pharmingen (Heidelberg, Germany). Recombinant human interleukin-4 (IL-4) and recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) were purchased from R&D Systems (Minneapolis, MN, USA). Lipolysaccharide (LPS) purified from *Escherichia coli* and Eosin Y were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rabbit anti-human OPN polyclonal antibody, anti-mouse and anti-rabbit horseradish peroxidase conjugated-immunoglobulin G were purchased from Abcam (Cambridge, MA, USA). Endotoxin levels in all agents were low (<1.0 EU/ml). The sources of other reagents are indicated in the text.

Preparation of curcumin and BPS. Curcumin was purchased from Enzo Life Sciences (Shanghai, China) and dissolved in ethyl alcohol. BPS was prepared in the Department of Pathology, Shandong University of Traditional Chinese Medicine (21) and dissolved in normal saline. The potential contamination of endotoxin in curcumin and BPS was detected using QCL-1000® Chromogenic LAL end-point assay (Lonza Walkersville, Inc., Walkersville, MD, USA), according to the manufacturer's instructions. The detection limit of the kit was 0.1 EU/ml. The endotoxin level of 50 μM curcumin and 100 μg/ml BPS preparations was <0.1 EU/ml.

Cell culture. The SKOV3 human ovarian cancer cell line was purchased from the Chinese Academy of Medical Sciences (Beijing, China). Cells were cultured in RPMI-1640 (Thermo Scientific HyClone, Logan, UT, USA), supplemented with 10% heat inactivated fetal bovine serum (FBS) (Thermo Scientific HyClone), 100 U/ml penicillin and 100 μg/ml streptomycin, at 37˚C in a 5% CO_2 humidified atmosphere. Curcumin (50 μM) or BPS (100 μg/ml) were added to the medium 24 h prior to experiments.

Generation of human monocyte-derived DCs. The use of human peripheral blood mononuclear cells (PBMCs) from healthy donors was approved by the Institutional Review Board of Shandong University (Jinan, China) and informed consent was obtained from the DC donors. Monocyte-derived DCs were prepared as previously described (22). Briefly, $CD14^+$ cells separated from PBMCs were enriched with a bead-labeled anti-CD14 monoclonal antibody using the magnetic antibody cell sorting system (Miltenyi Biotec, Bergisch-Gladbach, Germany). The purity of $CD14^+$ monocytes was >93%. $CD14^+$ monocytes were cultured for 5 days in complete RPMI medium containing granulocyte macrophage colony-stimulating factor (GM-CSF; 1,000 U/ml), IL-4 (500 U/ml) and curcumin (50 μM) or BPS (100 μg/ml). Cells were identified as immature DCs based on the positive expression of CD1a and CD209, the lack of CD14 and CD83 (purity >93%) and low expression of human leukocyte antigen (HLA)-DR, CD80 and CD86. To induce maturation, LPS (1 μg/ml) was added on day five and cells were cultured for a subsequent two days. Cell morphology and viability were determined by light microscopy (CKX31, Olympus, Tokyo, Japan) and flow cytometry (FACSCalibur; Becton Dickinson, San Jose, CA, USA). Cells were defined as mature DCs based on positive expression of CD1a, CD209, HLA-DR, CD83 and CD86 and a lack of CD14 (purity >93%).

Invasion assay. In preparation for the assay, a 24-well Transwell chamber with 8.0 μm pore size (CoStar, Cambridge, MA, USA) was pre-coated with 30 μg Matrigel (Becton Dickinson) diluted in phosphate-buffered saline. Cell suspensions ($1x10^5$ cells/well in serum-free growth media + 0.1% bovine serum albumin) were treated and added to the upper compartment of the insert. Media containing a chemoattractant (10% FBS) was added to the bottom chamber of the Transwell plates. Following incubation at 37˚C for 12 h, non-invaded cells (which remained on the upper surface of the filter) were removed and invaded cells (on the lower surface of the filter) were stained with Eosin Y and counted by light microscopy (Olympus CKX31, Tokyo, Japan). The number of migrating cells was adjusted by the cell viability assay to correct for possible toxic effects of curcumin or BPS treatment using the following equation: corrected migrating

cell number = counted migrating cell number/percentage of viable cells.

Quantitative polymerase chain reaction (qPCR). Total mRNA was extracted from cells by an RNeasy mini kit and purified by RNeasy mini spin columns (Qiagen Inc., Valencia, CA, USA), according to the manufacturer's instructions. cDNA synthesis was performed with oligo dT16 primers and Moloney murine leukemia virus reverse transcriptase according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). qPCR was performed on the LightCycler 2.0 instrument (Roche Diagnostics GmbH, Penzberg, Germany). Primer sequences were listed as follows: Forward: 5'-GGACAGCCAGGACTCCATTG-3' and reverse: 5'-TGTGGGGACAACTGGAGTGAA-3' for OPN; forward: 5'-CAGAGATGCGTGGAGAGTCG-3' and reverse: 5'-CAAAGGCGTCGTCAATCACC-3' for MMP-9, and, forward: 5'-AGCGAGCATCCCCCAAAGTT-3' and reverse: 5'-GGGCACGAAGGCTCATCATT-3' for β-actin. Gene-specific amplifications were demonstrated by melting-curve data.

Western blot analysis. A total of 20 μg protein (cell lysates) was subjected to electrophoresis in 10% sodium dodecyl sulfate-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (Immobilon™; Millipore, Bedford, MA, USA). Following transfer, gels were blocked with 5% skimmed milk in Tris-buffered saline with 0.05% Tween 20 (TBST) for 1 h, followed by overnight blotting with primary antibodies at 4˚C. Primary antibodies included a rabbit anti-human OPN polyclonal antibody (1:1,000) and a mouse anti-human β-actin mAb (1:1,000). Membranes were washed with TBST prior to incubation with secondary antibodies conjugated with horseradish peroxidase (1:2,000). An enhanced chemiluminescence system was used to detect horseradish peroxidase enzyme activity. Briefly, membranes were washed three times with buffer after the incubation with secondary antibodies and treated with enhanced chemilumescent substrates according to the manufacturer's instructions (Pierce Biotechnology, Inc., Rockford, IL, USA). The immunobands were visualized using the Kodak Digital Image Station IS2000 (Kodak, Rochester, NY, USA) and subsequently analyzed using densitometry with AlphaEaseFC software (version 4.0.0, Alpha Innotech Corp., Santa Clara, CA, USA).

Enzyme-linked immunosorbent assay (ELISA). OPN and MMP-9 in the cell supernatant were measured using ELISA kits (R&D Systems, Weisbaden, Germany), according to the manufacturer's instructions. Marker concentration was calculated from the standard curve. A subset of samples was assayed five times in every ELISA plate for quality control.

Flow cytometry. Surface receptor expression on SKOV-3 and DCs in the respective groups was detected using a FACSCalibur flow cytometer (Becton Dickinson). Two- or three-color immunofluorescence was performed using the following panel of fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- or PE-carbocyanin (Cy) 5 labeled monoclonal antibodies against CD14, CD1a, CD83, CD80, CD86, HLA-DR, CD209 and CD44. Isotype controls were run in parallel. Cell debris was eliminated from the analysis by forward and side scatter gating. For viability assays, cells were stained with Annexin V and propidium iodide, according to the manufacturer's instructions (Bender MedSystems, Vienna, Austria). The mean fluorescence intensities were determined by CellQuest software (Becton Dickinson).

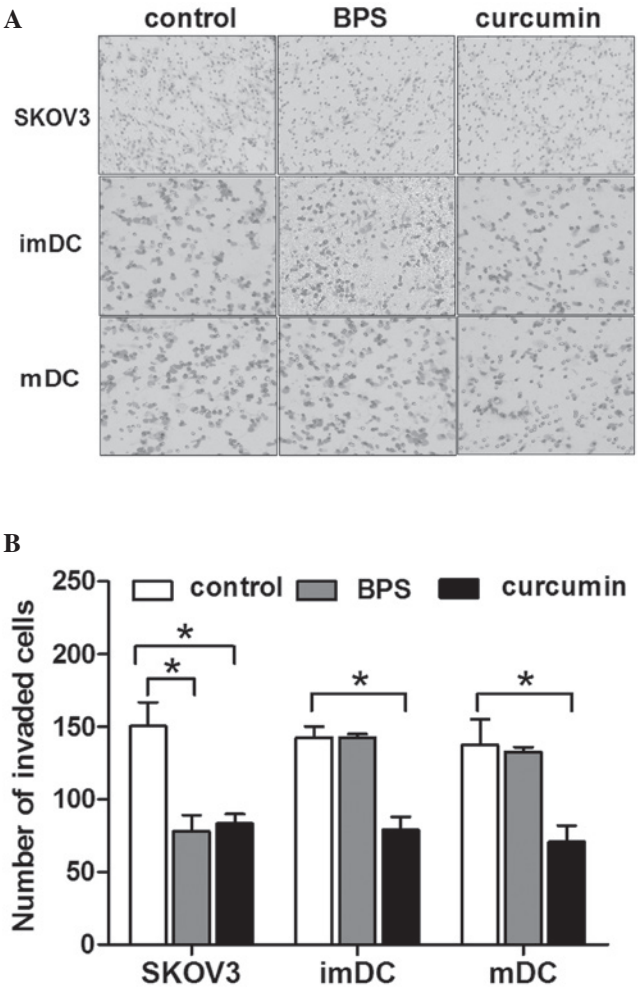

Figure 1. Effects of curcumin and BPS on invasion of SKOV3 cells and DCs. SKOV3 cells and imDCs or mDCs were treated with RPMI medium only (control), RPMI with curcumin (50 μM) or RPMI with BPS (100 μg/ml). Invasion was measured by Transwell. (A) One representative of three independent experiments was shown. (B) Invaded cell numbers of SKOV3 cells and imDCs or mDCs in the absence or presence of curcumin or BPS were counted. The data are presented as the mean ± SD (n=3, $^{*}P<0.05$). BPS, basil polysaccharide; DCs, dendritic cells; imDC, immature DCs; mDCS, mature DCs.

Statistical analysis. The SPSS software package (version 13.0; SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Data is expressed as the mean ± SD from at least three independent experiments. Statistical analysis was performed using a t-test or analysis of variance. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Distinct regulation of curcumin and BPS on invasion of SKOV3 cells and DCs. The regulation of BPS on the invasion

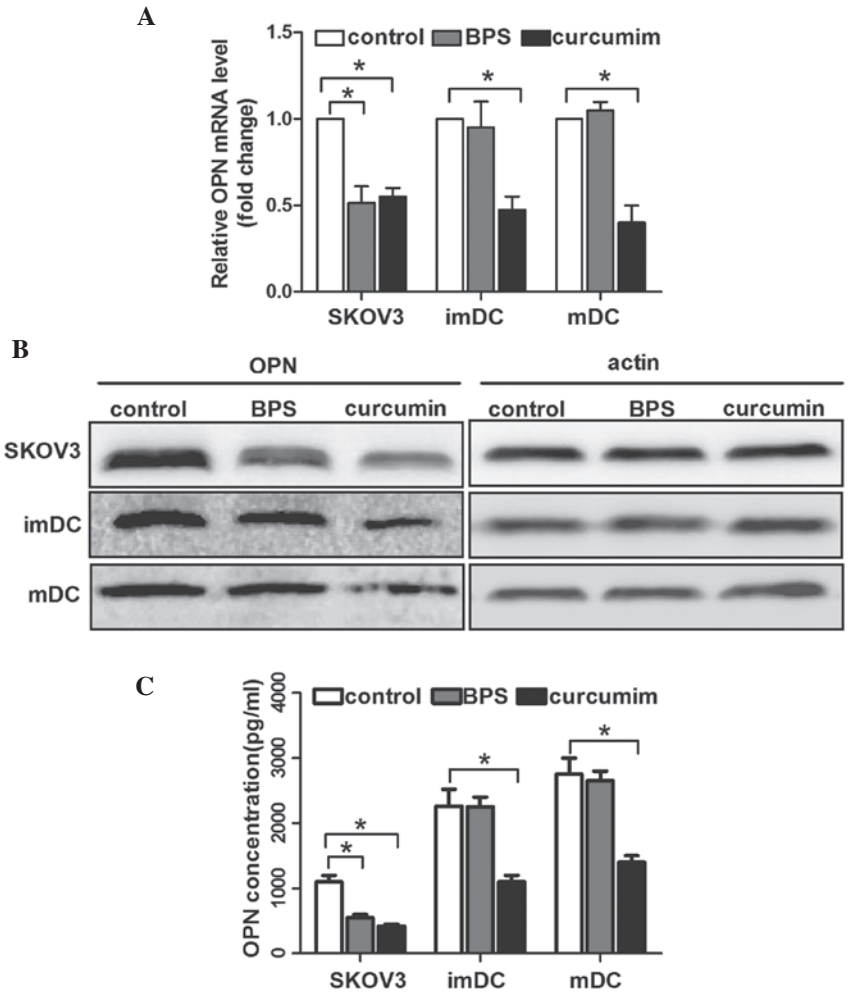

Figure 2. OPN expression of SKOV3 cells and DCs following curcumin or basil polysaccharide (BPS) treatment. SKOV3 cells, imDCs and mDCs were treated with RPMI medium only (control), RPMI with curcumin (50 μM) or RPMI with BPS (100 μg/ml) for certain time periods. (A) Expression of OPN mRNA was measured by qPCR analysis. The data are presented as the mean ± SD (n=3, *P<0.05). (B) Western blot analysis was used to evaluate OPN protein expression in cells. The image shown is representative of 3 independent experiments. (C) Enzyme-linked immunosorbent assay was used to analyze OPN levels in the cell supernatant. The data are presented as the mean ± SD (n=3, *P<0.05). OPN, Osteopontin; DCs, dendritic cells; BPS, basil polysaccharide; imDC, immature DCs; mDCS, mature DCs; qPCR, quantitative polymerase chain reaction.

of SKOV3 cells and DCs was investigated and compared with that of curcumin, as a number of previous studies have indicated that curcumin inhibited the motility of ovarian cancer cells and DCs (23-25). Results of the invasion assay showed that curcumin and BPS inhibited the invasion of SKOV3 cells and no difference was identified between the inhibitory efficiency of these two substances (Fig. 1). Curcumin significantly inhibited the invasion of immature and mature DCs, whereas the inhibitory effect was not exerted by BPS on immature or mature DCs (Fig. 1). These results indicated that BPS possesses the same inhibitory efficiency on the invasion of ovarian cancer cells as curcumin, but exerts minimal inhibition on DCs.

Curcumin and BPS alter the expression of OPN in SKOV3 cells and DCs. Previous studies have suggested that OPN promotes the motility of ovarian cancer cells and DCs (12,14,26). To investigate the underlying mechanisms of curcumin- and BPS-regulated cell invasion, mRNA and protein levels of OPN were measured. qPCR analysis showed that curcumin inhibited OPN mRNA expression in SKOV3 cells and immature and mature DCs, whereas BPS decreased OPN mRNA levels in SKOV3 cells, but not in immature or mature DCs (Fig. 2A). Consistent with its mRNA level, the protein expression and secretion of OPN regulated by curcumin in SKOV3 cells and DCs were significantly decreased; however, this decrease was only observed in BPS-treated SKOV3 cells and not in DCs (Fig. 2B and C). These results indicated that OPN expression was closely correlated with the curcumin- and BPS-regulated motility of ovarian cancer cells and DCs.

CD44 is downregulated in curcumin- but not BPS-treated SKOV3 cells and DCs. The CD44 surface expression on SKOV3 and DCs was analyzed following curcumin or BPS treatment by flow cytometry, as CD44 is an important receptor of OPN and it facilitates the invasion of cancer cells (8). Results of the current study showed that CD44 surface expression was significantly decreased in curcumin-treated SKOV3 cells and DCs, but its expression was not affected by BPS (Fig. 3). As well as decreasing OPN expression, curcumin was hypothesized to dampen its signaling activity by downregulating the functional receptor, CD44, in ovarian

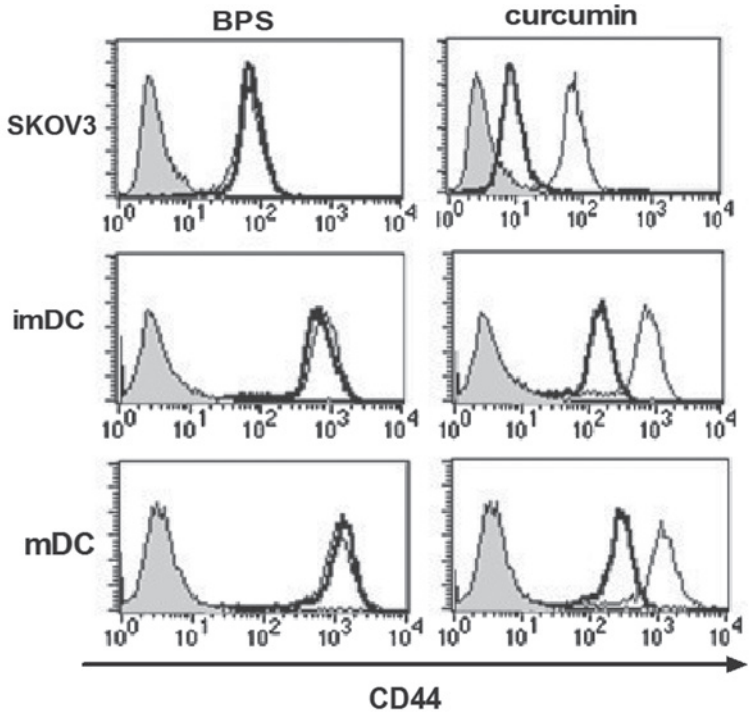

Figure 3. CD44 expression in SKOV3 cells and DCs following curcumin or BPS treatment. SKOV3 cells, imDCs and mDCs were treated with RPMI medium only (control), RPMI with curcumin (50 μM) or RPMI with BPS (100 μg/ml) for certain time periods. Expression of CD44 on cell surface was measured by flow cytometry. Isotype control (gray filled line), negative control (gray line) and curcumin or BPS treatment (black line) for CD44 are presented. Histograms are representative of at least three independent experiments. DCs, dendritic cells; BPS, basil polysaccharide; imDC, immature DCs; mDCS, mature DCs.

cancer cells and DCs. However, the effect of BPS on CD44 expression was not observed to be significant compared with that of curcumin.

MMP-9 is involved in curcumin- and BPS-modulated invasion of SKOV3 cells and DCs. OPN has been demonstrated to upregulate MMP-9 expression in the metastasis of a number of types of cancer (9-11). In addition, MMP-9 overexpression is markedly correlated with a higher risk of metastasis in ovarian cancer patients (27). Therefore, the effect of curcumin and BPS on MMP-9 expression of SKOV3 cells and DCs was investigated. qPCR results indicated that curcumin decreased MMP-9 mRNA expression in SKOV3 cells and DCs (Fig. 4A). By contrast, BPS decreased MMP-9 expression in SKOV3 cells, but not in immature or mature DCs (Fig. 4A). Consistent with its mRNA level, ELISA results indicated that the concentration of MMP-9 in the supernatant of curcumin-treated SKOV3 cells and DCs was significantly lower, compared with the control group. BPS decreased MMP-9 concentration in the supernatant of SKOV3 cells, but the MMP-9 level in the supernatant of DCs was not affected (Fig. 4B). This correlation between MMP-9 expression and invasion activity suggested that the alteration of MMP-9 expression was involved in curcumin and BPS modulated invasion of SKOV3 cells and DCs.

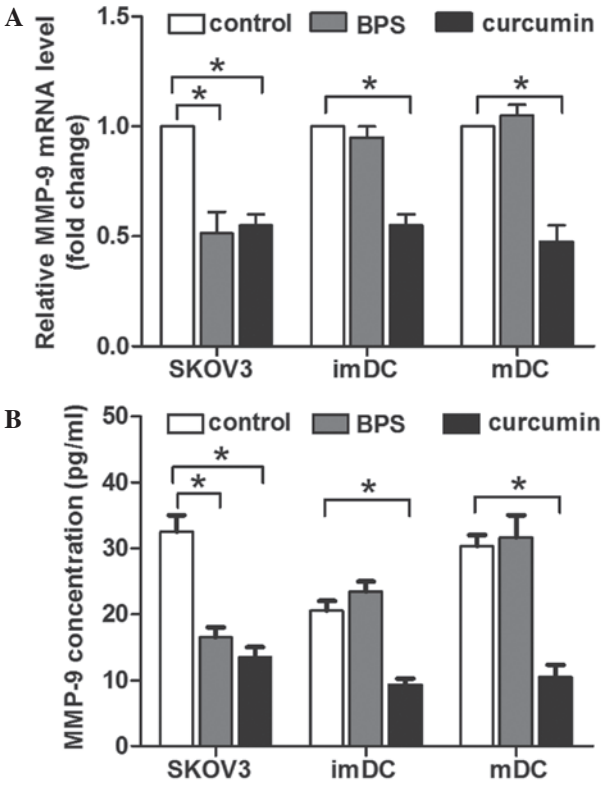

Figure 4. MMP-9 expression in SKOV3 cells and DCs following curcumin or BPS treatment. SKOV3 cells, imDCs and mDCs were treated with RPMI medium only (control), RPMI with curcumin (50 μM) or RPMI BPS (100 μg/ml) for certain time periods. Levels of MMP-9 was measured by (A) quantitative polymerase chain reaction and (B) enzyme-linked immunosorbent assay. The data are shown as the mean ± SD (n=3, *P<0.05). MMP-9, matrix metallopeptidase-9; DCs, dendritic cells; BPS, basil polysaccharide; imDC, immature DCs; mDCS, mature DCs.

Discussion

In the current study, the regulation of two natural products, curcumin and BPS, on the invasion of SKOV3 ovarian cancer cells and DCs, and the underlying mechanisms were

investigated. The observations indicated that curcumin impeded the invasion of SKOV3 cells and immature or mature DCs. Compared with curcumin, BPS showed similar inhibitory efficiency on SKOV3 cell invasion, but its effect on DCs was minimal. Further investigation showed that curcumin decreased the levels of OPN, CD44 and MMP-9 expression on the surface of ovarian cancer cells and DCs, while BPS decreased OPN and MMP-9 expression on ovarian cancer cells and showed no inhibitory effect on CD44 expression. Therefore, these results indicated that distinct regulation of OPN, CD44 and MMP-9 expression was at least partly responsible for the motility change of two types of cells mediated by curcumin or BPS.

The therapeutic effect of curcumin on ovarian cancer has been well established by *in vitro* and *in vivo* studies (16,28-30). Consistent with previous studies, which have demonstrated that curcumin decreased ovarian cancer cell migration (23,24), the current study confirmed that it significantly inhibited the invasion of SKOV3 cells, which provided more evidence for its anticancer mechanisms. However, the effect of curcumin on the immune system appears to be controversial, primarily dependent on different types of immunocytes. Although curcumin was shown to directly enhance T cell-mediated antitumor immunity, it inhibited the antigen-presenting properties of DCs by blocking maturation marker expression and inducing differentiation towards a tolerogenic phenotypes (25,31,32). In the current study, curcumin was observed to inhibit the invasion of immature and mature DCs. Compared with curcumin, BPS appears more beneficial as a complementary therapy in ovarian cancer treatment, as the results showed that it inhibited SKOV3 cell invasion, almost as efficiently as curcumin, whereas no inhibition was observed on the invasion of immature or mature DCs. These results indicated that BPS may achieve more robust antitumor immunity in ovarian cancer patients than curcumin as the invasion of activated DCs to secondary lymph organs is vital for the subsequent excitation of T cells. However, in addition to motility, the immune-initiating potency of DCs is also dependent upon its phagocytotic activity, surface molecule expression and cytokine secretion. Therefore, this hypothesis may not be confirmed until further studies regarding the modulation of BPS on DCs biological properties are conducted.

OPN enhances cell invasion by interacting with integrins and CD44 and initiating subsequent reactions (12,14,26), for example, enhancing MMP secretion (9,11). In the present study, curcumin was observed to inhibit OPN expression in SKOV3 cells and DCs, while BPS decreased OPN expression in SKOV3 cells, but not in DCs. The correlation between the levels of OPN and cell motility indicated that curcumin and BPS modulated SKOV3 cells and DC invasion by altering the expression of OPN. The results indicated that the protein level and secretion of OPN was significantly affected following treatment wotj curcumin or BPS and a significant decrease of OPN mRNA may be responsible for this change in protein levels. However, more modifications may have occurred at the translational or post-translational level after the mRNA was formed, such as methylation, phosphorylation and acetylization. All these modulations would affect the protein synthesis of OPN, but their effects were not investigated in the present study. In addition, the distinct effect of BPS on OPN expression in cancer cells and DCs is noteworthy. This may be explained by various activation pathways of OPN expression in cancer cells and DCs; however, further studies are required to investigate the underlying mechanisms.

Cellular adhesion molecules (CAMs) mediate contact among or between cells and the ECM, and dysregulation of their expression is involved in tumor progression. CD44 has been shown to be involved in cancer metastasis (33). In addition, the close correlation between CD44 and OPN-mediated cell invasion has been well described as OPN was observed to upregulate CD44 surface expression (34) and CD44 is known to promote cell invasion by binding with hyaluronan in ECM. However, OPN directly collaborates with CD44 to activate downstream signaling pathways in an autocrine manner and subsequently promotes cell invasion and chemotaxis (35,36). The current study demonstrated that curcumin significantly downregulated the expression of CD44 on the surface of SKOV3 cells and DCs, while BPS had no marked effect on CD44 expression. These results indicated that curcumin not only decreased OPN expression, but also dampened its activity by inhibiting the expression of its functional receptor.

OPN induced MMP expression, was involved in the metastasis of cancer cells (9,11) and curcumin was shown to inhibit the expression of MMP-2 by inactivating the OPN-mediated nuclear factor-κB (NF-κB) pathway (37). Curcumin and BPS were observed to modulate MMP-9 expression in a manner consistent with the OPN level. This was achieved by decreasing the OPN level or directly interfering with OPN-MMP-9 pathway activity or a combination of the two. Moreover, activation of CD44 promotes the binding of MMP-9 to the cell surface and its maturation (38), and CD44-MMP-9 aggregates, enhanced cancer metastasis in a murine mammary carcinoma model (39). Although curcumin and BPS decreased MMP-9 secretion in SKOV3 cells to a similar efficacy, the maturation of MMP-9 may differ, given that curcumin significantly downregulated CD44 expression when compared with BPS. The current results showed that curcumin and BPS modulated MMP-9 secretion in a manner that was consistent with cell invasion and OPN expression, thereby it may be a potential mechanism for the modulation of cell motility.

In conclusion, to the best of our knowledge, this was the first study to demonstrate that BPS inhibited the invasion of SKOV3 cells while curcumin affected SKOV3 cells and DCs. This diversity was achieved, in part, by the distinct expression of OPN, CD44 and MMP-9 in the two types of cells. The present study indicated that curcumin and BPS may serve as efficient complementary therapies for ovarian cancer. Notably however, BPS may be a superior candidate for maintaining anticancer immunity activity in ovarian cancer patients, considering its low inhibitory effect on DC invasion. However, further investigation is required to investigate whether these compounds are equally effective *in vivo*.

Acknowledgements

This study was supported by a grant from the National Natural Science Foundation of China (grant nos. 30472261, 31270971 and 81072406).

References

1. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2012. CA Cancer J Clin 62: 10-29, 2012.
2. Amati L, Pepe M, Passeri ME, Mastronardi ML, Jirillo E and Covelli V: Toll-like receptor signaling mechanisms involved in dendritic cell activation: potential therapeutic control of T cell polarization. Curr Pharm Des 12: 4247-4254, 2006.
3. Chu CS, Boyer J, Schullery DS, *et al*: Phase I/II randomized trial of dendritic cell vaccination with or without cyclophosphamide for consolidation therapy of advanced ovarian cancer in first or second remission. Cancer Immunol Immunother 61: 629-641, 2012.
4. Triozzi PL, Khurram R, Aldrich WA, Walker MJ, Kim JA and Jaynes S: Intratumoral injection of dendritic cells derived in vitro in patients with metastatic cancer. Cancer 89: 2646-2654, 2000.
5. Chakraborty G, Jain S, Behera R, *et al*: The multifaceted roles of osteopontin in cell signaling, tumor progression and angiogenesis. Curr Mol Med 6: 819-830, 2006.
6. Bulfone-Paus S and Paus R: Osteopontin as a new player in mast cell biology. Eur J Immunol 38: 338-341, 2008.
7. Pagano PJ and Haurani MJ: Vascular cell locomotion: osteopontin, NADPH oxidase, and matrix metalloproteinase-9. Circ Res 98: 1453-1455, 2006.
8. Khan SA, Cook AC, Kappil M, *et al*: Enhanced cell surface CD44 variant (v6, v9) expression by osteopontin in breast cancer epithelial cells facilitates tumor cell migration: novel post-transcriptional, post-translational regulation. Clin Exp Metastasis 22: 663-673, 2005.
9. Chen YJ, Wei YY, Chen HT, *et al*: Osteopontin increases migration and MMP-9 up-regulation via alphavbeta3 integrin, FAK, ERK, and NF-kappaB-dependent pathway in human chondrosarcoma cells. J Cell Physiol 221: 98-108, 2009.
10. Desai B, Ma T, Zhu J and Chellaiah MA: Characterization of the expression of variant and standard CD44 in prostate cancer cells: identification of the possible molecular mechanism of CD44/MMP9 complex formation on the cell surface. J Cell Biochem 108: 272-284, 2009.
11. Yang G, Zhang Y, Wu J, *et al*: Osteopontin regulates growth and migration of human nasopharyngeal cancer cells. Mol Med Rep 4: 1169-1173, 2011.
12. Schulz G, Renkl AC, Seier A, Liaw L and Weiss JM: Regulated osteopontin expression by dendritic cells decisively affects their migratory capacity. J Invest Dermatol 128: 2541-2544, 2008.
13. Visintin I, Feng Z, Longton G, *et al*: Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res 14: 1065-1072, 2008.
14. Bao LH, Sakaguchi H, Fujimoto J and Tamaya T: Osteopontin in metastatic lesions as a prognostic marker in ovarian cancers. J Biomed Sci 14: 373-381, 2007.
15. Zhang X, Zhang HQ, Zhu GH, *et al*: A novel mono-carbonyl analogue of curcumin induces apoptosis in ovarian carcinoma cells via endoplasmic reticulum stress and reactive oxygen species production. Mol Med Rep 5: 739-744, 2012.
16. Selvendiran K, Ahmed S, Dayton A, *et al*: HO-3867, a curcumin analog, sensitizes cisplatin-resistant ovarian carcinoma, leading to therapeutic synergy through STAT3 inhibition. Cancer Biol Ther 12: 837-845, 2011.
17. Sadzuka Y, Nagamine M, Toyooka T, Ibuki Y and Sonobe T: Beneficial effects of curcumin on antitumor activity and adverse reactions of doxorubicin. Int J Pharm 432: 42-49, 2012.
18. Luo F, Song X, Zhang Y and Chu Y: Low-dose curcumin leads to the inhibition of tumor growth via enhancing CTL-mediated antitumor immunity. Int Immunopharmacol 11: 1234-1240, 2011.
19. Bhattacharyya S, Md Sakib Hossain D, Mohanty S, *et al*: Curcumin reverses T cell-mediated adaptive immune dysfunctions in tumor-bearing hosts. Cell Mol Immunol 7: 306-315, 2010.
20. Badisa RB, Tzakou O, Couladis M and Pilarinou E: Cytotoxic activities of some Greek Labiatae herbs. Phytother Res 17: 472-476, 2003.
21. Xia L, Zheng G, Zhou H, *et al*: The application of BPS in the preparing of anti-metastasis therapies. P.R. China Patent CN1498625. Filed, November 8, 2002; Issued, May 26, 2004 (In Chinese).
22. Renkl AC, Wussler J, Ahrens T, *et al*: Osteopontin functionally activates dendritic cells and induces their differentiation toward a Th1-polarizing phenotype. Blood 106: 946-955, 2005.
23. Ji C, Cao C, Lu S, *et al*: Curcumin attenuates EGF-induced AQP3 up-regulation and cell migration in human ovarian cancer cells. Cancer Chemother Pharmacol 62: 857-865, 2008.
24. Seo JH, Jeong KJ, Oh WJ, *et al*: Lysophosphatidic acid induces STAT3 phosphorylation and ovarian cancer cell motility: their inhibition by curcumin. Cancer lett 288: 50-56, 2010.
25. Shirley SA, Montpetit AJ, Lockey RF and Mohapatra SS: Curcumin prevents human dendritic cell response to immune stimulants. Biochem Biophys Res Commun 374: 431-436, 2008.
26. Tilli TM, Franco VF, Robbs BK, *et al*: Osteopontin-c splicing isoform contributes to ovarian cancer progression. Mol Cancer Res 9: 280-293, 2011.
27. Zhang W, Yang HC, Wang Q, *et al*: Clinical value of combined detection of serum matrix metalloproteinase-9, heparanase, and cathepsin for determining ovarian cancer invasion and metastasis. Anticancer Res 31: 3423-3428, 2011.
28. Lin YG, Kunnumakkara AB, Nair A, *et al*: Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. Clin Cancer Res 13: 3423-3430, 2007.
29. Watson JL, Greenshields A, Hill R, *et al*: Curcumin-induced apoptosis in ovarian carcinoma cells is p53-independent and involves p38 mitogen-activated protein kinase activation and downregulation of Bcl-2 and survivin expression and Akt signaling. Mol Carcinog 49: 13-24, 2010.
30. Ganta S, Devalapally H and Amiji M: Curcumin enhances oral bioavailability and anti-tumor therapeutic efficacy of paclitaxel upon administration in nanoemulsion formulation. J Pharm Sci 99: 4630-4641, 2010.
31. Rogers NM, Kireta S and Coates PT: Curcumin induces maturation-arrested dendritic cells that expand regulatory T cells in vitro and in vivo. Clin Exp Immunol 162: 460-473, 2010.
32. Cong Y, Wang L, Konrad A, Schoeb T and Elson CO: Curcumin induces the tolerogenic dendritic cell that promotes differentiation of intestine-protective regulatory T cells. Eur J Immunol 39: 3134-3146, 2009.
33. Orian-Rousseau V: CD44, a therapeutic target for metastasising tumours. Eur J Cancer 46: 1271-1277, 2010.
34. Samanna V, Wei H, Ego-Osuala D and Chellaiah MA: Alpha-V-dependent outside-in signaling is required for the regulation of CD44 surface expression, MMP-2 secretion, and cell migration by osteopontin in human melanoma cells. Exp Cell Res 312: 2214-2230, 2006.
35. Jijiwa M, Demir H, Gupta S, *et al*: CD44v6 regulates growth of brain tumor stem cells partially through the AKT-mediated pathway. PloS One 6: e24217, 2011.
36. Chagan-Yasutan H, Tsukasaki K, Takahashi Y, *et al*: Involvement of osteopontin and its signaling molecule CD44 in clinicopathological features of adult T cell leukemia. Leuk Res 35: 1484-1490, 2011.
37. Philip S and Kundu GC: Osteopontin induces nuclear factor kappa B-mediated promatrix metalloproteinase-2 activation through I kappa B alpha /IKK signaling pathways, and curcumin (diferulolylmethane) down-regulates these pathways. J Biol Chem 278: 14487-14497, 2003.
38. Bagnoli F, Oliveira VM, Silva MA, Taromaru GC, Rinaldi JF and Aoki T: The interaction between aromatase, metalloproteinase 2,9 and CD44 in breast cancer. Rev Assoc Med Bras 56: 472-477, 2010.
39. Yu Q and Stamenkovic I: Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 14: 163-176, 2000.