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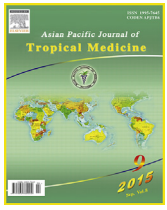

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Effect of *BRMS1* expression on proliferation, migration and adhesion of mouse forestomach carcinoma

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ABSTRACT

Objective: To discuss the effect of *BRMS1* on the proliferation, migration and adhesion of mouse forestomach carcinoma.

Methods: The constructed pCMV-myc-*BRMS1* recombinant plasmid and blank plasmid were transfected into mouse forestomach carcinoma. MTT method was employed to measure the activity of gastric cancer cell; the scratch assay and Transwell assay to measure the migration and invasion of gastric cancer cell; the adhesion assay to measure the adhesion of gastric cancer cell; while the Western blot assay to measure the expression of The NF-κB signal pathway, downstream matrix metalloproteinase (MMP)-2, MMP-9 and osteopontin and E-cadherin in the gastric cancer cell. Besides, the transplanted animal model of gastric cancer in mice was constructed to measure the size of tumor xenograft.

Results: Results of MTT assay showed that, compared with the empty vector control group, the activity of gastric cancer cell was not affected in the *BRMS1* transfection group. The improved expression of *BRMS1* could inhibit the adhesion, migration and invasion of gastric cancer cell ($P < 0.01$). Besides, compared with the empty vector control group, the phosphorylation of NF-κB p65 and IκBα was reduced in the *BRMS1* transfection group, with the decreased expression of MMP-2, MMP-9 and osteopontin and the increased expression of E-cadherin ($P < 0.01$). Results of animal experiment also showed that the expression of *BRMS1* did not affect the transplanted tumor.

Conclusions: The expression of *BRMS1* can significantly inhibit the adhesion, migration, invasion and metastasis of mouse forestomach carcinoma gastric cancer cell, which is related to The NF-κB signal pathway.

1. Introduction

The tumor metastasis suppressor gene is some kind of gene related to the tumor, with the high expression in the non-metastatic tumor and low expression in the metastatic one. As it does not affect the growth of tumor, it is regarded as the one to inhibit the metastasis of tumor. The breast cancer metastasis suppressor 1 (*BRMS1*) is the tumor metastasis suppressor gene that was found on human chromosome 11 in 2000. The subsequent researches also indicated the low expression of *BRMS1* gene in many tumors such as the breast cancer, malignant melanoma and bladder cancer, with the certain inhibition against the metastasis of tumor [1,2]. For instance, Cicek *et al.* [1] transfected *BRMS1* into MDA-MB-231 and MDA-MB-435 of breast cancer cells, which could reduce the performance of metastasis by 50%–90%. Samant *et al.* [2] also transfected *BRMS1* into MDA-MB-231 of breast cancer cells to reduce the performance of metastasis by 70%–90%. Meanwhile, it was also found that when injecting the MDA-MB-231 and MDA-MB-435 of breast cancer cells with the transfected *BRMS1* gene into the nude mouse mammary

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fat pad, the metastasis of its lung and regional lymph node was significantly reduce, which fully showed that the expression of *BRMS1* could deeply affect the metastasis of tumor.

Liu *et al.* [3] transfected *BRMS1* shRNAs into the non-small cell lung cancer, which could promote the migration of cells and enhance the transcriptional activity of NF-κB RelA/p65. Cicek *et al.* [4] transfected *BRMS1* into the human melanoma cell line C8161.9 and found the negative correlation between the expression of *BRMS1* and binding activity of NF-κB. *BRMS1* could rely on The NF-κB signal pathway to regulate the expression of OPN and thus affect the metastasis of breast cancer and the tumor of animal model of glioma [5]. Sheng *et al.* [6] performed the experiments of RT-PCR and WB and found that the knockout of *BRMS1* from OVCAR3 of breast cancer cells could significantly improve the migration and invasion of cells, which was related to the improved expression of NF-κB p65 and osteopontin. The overexpression of *BRMS1* in glioma cells could significantly down-regulate the expression of NF-κB and inhibit the expression and activity of matrix metalloproteinase (MMP)-2 and MMP9 and thus inhibit the adhesion, migration and invasion of cancer cells [7]. In the gastric cancer cell SGC-7901 and transplanted tumor model of gastric cancer, the activity of NF-κB was negatively relative to the expression of E-cadherin [8,9]. It thus indicated that *BRMS1* could affect the process of migration, invasion, adhesion and metastasis of tumor cells through The NF-κB signal pathway and then regulate the expression of related proteins.

The expression of *BRMS1* was significantly reduced or lost in the gastric cancer, which was negatively related to the metastasis of lymph node [10]. But the specific mechanism of inhibition against the metastasis of gastric cancer has been unknown. Under such background, we constructed the mouse forestomach carcinoma strains with the overexpression of *BRMS1* and then performed MTT assay and experiments of adhesion, migration and invasion to discuss the effect of expression of *BRMS1* on the proliferation, adhesion, migration and invasion of mouse forestomach carcinoma gastric cancer cell strains. Besides, Western blot assay was employed to measure The NF-κB signal pathway and the expression of downstream proteins MMP-2, MMP-9, osteopontin, and E-cadherin. Finally, the transplanted animal model of gastric cancer in mice was constructed to measure the effect of expression of *BRMS1* on the size of transplanted tumor.

2. Materials and methods

2.1. Materials and reagents

The mouse forestomach carcinoma was purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences, with the item No. of 31800022; the tetrazolium salt (MTT) from Sigma; the rabbit anti MMP-2, MMP-9, osteopontin and E-cadherin antibodies from Epitomics; the rabbit anti NF-κB p65, p-NF-κB p65, IκBα and p-IκBα antibodies from Cell Signaling Technology; the Transwell chamber from Corning; the fetal bovine serum, RPMI-1640 medium and trypsin from Gbico; the crystal violet from Sigma; CO_2 incubator from Thermo Scientific; the inverted microscope from Nikon; dual mini vertical electrophoresis apparatus, mini transfer electrophoresis apparatus and ChemiDoc™ XRS system from Bio-Rad.

2.2. Construction of BRMS1 eukaryotic expression vector

The gene sequence of *BRMS1* was searched in GeneBank and then the primer was designed with the sequence: upstream 5′-ATGCCTGTCCAGCCTCCAAG-3′, 5′-GCGTCGCTCA-TAGTCCTCATCA-3′. The target fragment was extracted using Trizol method, while PCR for the amplification of target fragment and electrophoresis for the detection of target product. The double digestion and link was performed, being followed by the plasmid transformation and extraction, and then PCR screening, identification by restriction analysis and sequencing, finally obtain the *BRMS1* eukaryotic expression vector.

2.3. Measurement of cell proliferation rate using MTT method

Cells were seeded onto 96-well plate, with 200 μL in each well respectively. When the confluency of cells reached to 70%, it was to transfect pCMV-myc-*BRMS1* recombinant plasmid and blank plasmid. 48 h after the transfection, 20 μL 5 mg/mL MTT was added for the continuous culture of 4 h and then the culture medium was sucked out. Afterwards, 150 μL DMSO was added in each well and then it was shaken to fully dissolve the crystals. CD value was measured at 560 nm of analyzer of enzyme-linked immunosorbent assay, while 630 nm was chosen as the reference wavelength to calculate the proliferation rate.

2.4. Scratch assay

Cells were seeded onto 6-well plate. When the confluency of cells reached to 70%, it was to transfect pCMV-myc-*BRMS1* recombinant plasmid and blank plasmid respectively. 48 h after the transfection, 20 μL pipette gun head was employed to scratch on 6-well plate. It was then washed by PBS for three times. Afterwards, the serum-free RPMI-1640 was chosen for the culture, which was observed under Nikon inverted microscope. The scratch width was measured and photographed.

2.5. Cell adhesion assay

48 h after the cell culture with the terminated transfection, each group of cells was collected. The cell concentration was adjusted to 1×10^5, which was seeded on 96-well plate that was coated by Matrigel (50 μg/mL). Inadhesive cells were washed off, while the absorbance value of each well at 570 nm was measured using MTT method. The optical density could be used to reflect the ratio of cells adhered to Matrigel on 96-well plate, with the calculation equation: Optical density value of cells adhered to Matrigel/optical density value of total cells × 100%.

2.6. Cell invasion assay

Matrigel basement membrane was evenly paved on the micromembrane (5 μm) of Transwell chamber to be prepared as

the gel for further use. Cells were seeded onto 96-well plate, with 100 μL in each well. When the confluency of cells reached to 70%, it was to transfect pCMV-myc-*BRMS1* recombinant plasmid and blank plasmid. 48 h after the transfection, cells were digested and added in the upper chamber of Transwell, while the lower chamber contained DEME medium with 5% fetal bovine serum for the further 24 h of culture. Then the Transwell chamber was taken out and washed, using the paraformaldehyde for the fixation. It was then stained with the crystal violet and the stained cytoplasm appeared to be purple. The number of membrane-penetrating cells in five fields was counted under the inverted optical microscope and the mean number of cells for each field was calculated to represent the invasion ability of cells.

2.7. Western blot assay

Cells were seeded onto 96-well plate, with 100 μL in each well. When the confluency of cells reached to 70%, it was to transfect pCMV-myc-*BRMS1* recombinant plasmid and blank plasmid. 48 h after the transfection, cells were scraped and centrifuged. Then RIPA lysis buffer was added with the certain quantity. It was put in Vortex instrument for 30 s of shaking every 10 min. After 40 min, it was centrifuged at 4 °C and 10000 rpm for 10 min. The supernatant was sucked carefully to obtain the total protein. The protein concentration was measured with BCA kit. The protein loading buffer was treated with SDS gel electrophoresis and then it was transferred with the wet method. Then the film was immersed into the primary antibody solution for the incubation at 4 °C over night. After being washed, it was immersed into the secondary antibody solution (1:100) for the incubation at the room temperature for 1–2 h. Afterwards, the film was taken out and washed, while ECL reagent was added on the film for the exposure in the gel imaging system. Statistics was performed on the gray value of each antibody band using 'Quantity one' software.

2.8. Nude mice test

40 nude mice were divided into 2 groups, with 20 subjects in each one respectively. The empty vector control group was injected with the gastric cancer cells that contained the blank plasmid, while the *BRMS1* transfection group was injected with the gastric cancer cells that contained the *BRMS1* recombinant plasmid. On the day before the cell transfection, cells were seeded on 10 cm culture dish, with 12 dishes in total. On the next day, it was to transfect pCMV-myc-*BRMS1* recombinant plasmid and blank plasmid. Cells were collected after 24 h. The cell concentration was regulated to 3×10^6 cells per 100 μL. Afterwards, it was transferred to a sterile EP tube and then injected into right skin of nude mice. Beginning from the 7th day, the size of tumor was measured every 3 days, which was expressed as the tumor volume.

2.9. Data analysis

The results were expressed as mean ± SD, with three repeats for each set of data at least. The *t* test was employed and $P < 0.05$ was meant to be significant difference. All data were analyzed using SPSS 17.0.

3. Results

3.1. Effect of BRMS1 on activity of mouse forestomach carcinoma

There was no significant difference in the activity of gastric cancer cells between *BRMS1* transfection group and empty vector control group ($P > 0.05$), with the value of 1.00 ± 0.12 and 0.96 ± 0.05, respectively.

3.2. Effect of BRMS1 on adhesion of mouse forestomach carcinoma

As shown in Figure 1, according to MTT assay, compared with the empty vector control group, the adhesion of gastric cancer cells in *BRMS1* transfection group was reduced, with the statistical significant difference ($P < 0.01$).

3.3. Effect of BRMS1 on migration and invasion of mouse forestomach carcinoma

As shown in Figure 2, the migration distance of cells in *BRMS1* transfection group and empty vector control group was (32.48 ± 2.09) μm and (52.38 ± 2.57) μm respectively, with the statistical difference ($P < 0.01$). As shown in Figure 3, the number of gastric cancer cells that penetrated through the lower chamber in *BRMS1* transfection group and empty vector control group was 59.68 ± 9.27 and 99.37 ± 8.02 respectively, with the statistical significant difference ($P < 0.01$).

3.4. Effect of BRMS1 on expression of MMPs in mouse forestomach carcinoma

After transfecting *BRMS1* recombinant plasmid and blank plasmid in the mouse forestomach carcinoma, results showed that, compared with the control group, the expression of MMP-2 and MMP-9 was significantly down-regulated in *BRMS1* transfection group, with the statistical significant difference ($P < 0.01$), as shown in Figure 4.

3.5. Effect of BRMS1 on expression of osteopontin and E-cadherin in mouse forestomach carcinoma

After transfecting *BRMS1* recombinant plasmid and blank plasmid in the mouse forestomach carcinoma, results showed that, compared with the control group, the expression of osteopontin was down-regulated and the expression of E-cadherin

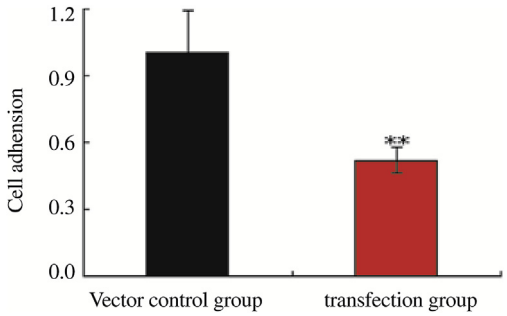

Figure 1. Effect of *BRMS1* on adhesion of mouse forestomach carcinoma. Compared with the empty vector control group, $P < 0.01$.

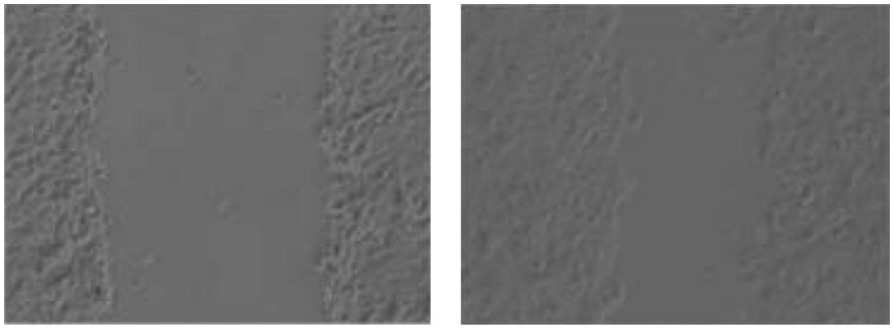

Figure 2. Effect of *BRMS1* on migration of mouse forestomach carcinoma.
A: Empty vector control group, B: *BRMS1* transfection group.

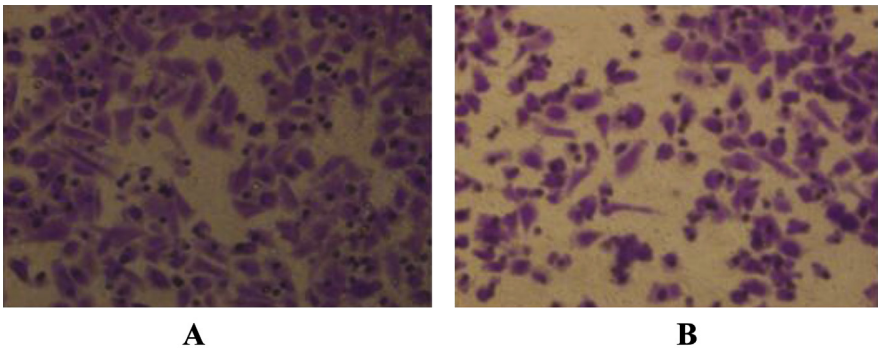

Figure 3. Effect of *BRMS1* on invasion of mouse forestomach carcinoma.
A: Empty vector control group, B: *BRMS1* transfection group.

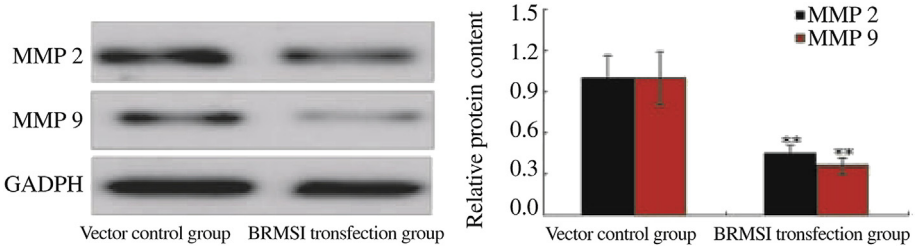

Figure 4. Effect of *BRMS1* on expression of MMPs in mouse forestomach carcinoma.
Compared with the empty vector control group, $P < 0.01$.

was up-regulated in *BRMS1* transfection group, with the statistical significant difference ($P < 0.01$), as shown in Figure 5.

3.6. Effect of BRMS1 on NF-κB signal pathway in mouse forestomach carcinoma

After transfecting *BRMS1* recombinant plasmid and blank plasmid in the mouse forestomach carcinoma, results showed that, compared with the control group, the phosphorylation of NF-κB p65 and IκBα was reduced in *BRMS1* transfection group, with the statistical significant difference ($P < 0.01$), as shown in Figure 6.

3.7. Effect of BRMS1 on size of transplanted tumor of nude mice with gastric cancer

After the subcutaneous injection of mouse forestomach carcinoma gastric cancer cells, the tumor size was measured since the 7th day and every 3 days. Except the 13th and 19th days,

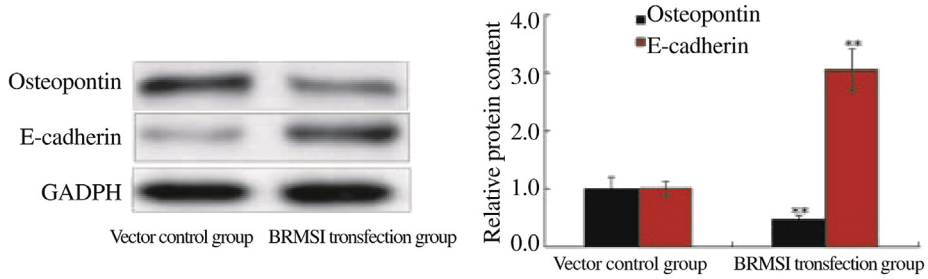

Figure 5. Effect of *BRMS1* on expression of osteopontin and E-cadherin in mouse forestomach carcinoma.
Compared with the empty vector control group, $P < 0.01$.

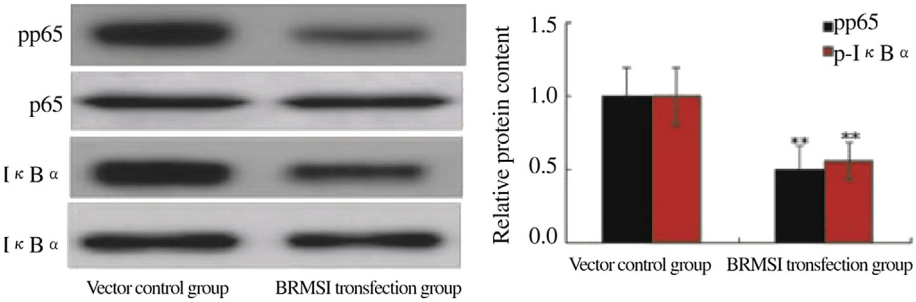

Figure 6. Effect of *BRMS1* on NF-κB signal pathway in mouse forestomach carcinoma. Compared with the empty vector control group, $P < 0.01$.

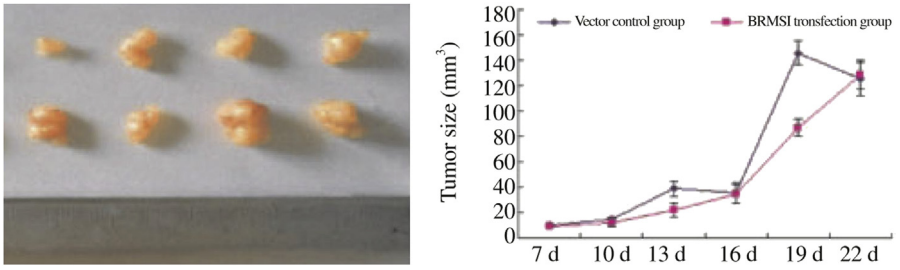

Figure 7. Effect of *BRMS1* on size of transplanted tumor of nude mice with gastric cancer.

compared with the empty vector control group, there was no significant change in the size of transplanted tumor in *BRMS1* transfection group, as shown in Figure 7 (the left figure began from the 13th day).

4. Discussion

When transfecting *BRMS1* in the human melanoma cell line C8161.9, results showed that the tumor formation of melanoma cell was not affected, but the metastasis of melanoma was significantly inhibited [11]. Furthermore, when *BRMS1* plasmid was transfected into epithelial ovarian cancer cell lines HO-8910PM, results showed that the expression of *BRMS1* would not affect the growth of cancer cells *in vitro* or primary tumor *in vivo*, but it could significantly inhibit the adhesion of cancer cells and extracellular matrix [12]. *BRMS1* was transfected in the lung cancer cells NCI-H1299 and thus the animal model of lung cancer was constructed. Results of the *in vitro* and *in vivo* tests all showed that *BRMS1* could significantly inhibit the metastasis of NCI-H1299 cells, but not affect the growth of primary tumor [13]. This study also proved that the expression of *BRMS1* would not affect the activity of mouse forestomach carcinoma cells or change the size of transplanted gastric cancer. It indicated that the expression of *BRMS1* could significantly affect the invasion and metastasis of cancer cells, but not affect its growth, while the specific mechanism had been unknown. The key step to the dynamic process of invasion and metastasis of tumor cells was how to penetrate through the biological containments. The adhesion molecule was to mediate the adhesion of cancer cells and basement membrane or extracellular matrix and then induce the tumor cells to release or activate multiple proteolytic enzymes to degrade the basement membrane and extracellular matrix. Finally, under the combined action of multiple cytokines, it was migrated to the target tissue for the clonal proliferation to form the metastasis. Therefore, it could also be regarded that *BRMS1* could inhibit the adhesion, migration and invasion of tumor cells and thus inhibit the metastasis of tumor.

NF-κB is the transcription factor of cell nucleus, which is closely related to the apoptosis, invasion and metastasis of tumor cells. In case of the external stimulation, it can be separated from IκB and then be transplanted in the nucleus to be integrated with the target gene, which could play the role of transcription and regulation, drive the occurrence of molecular mechanisms related to the adhesion, invasion and metastasis of tumor, regulate the expression of proteins of E-cadherin, osteopontin and MMPs and thus promote the mobility and invasion of cancer cells. According to Long *et al.* [14], the expression of NF-κB was decreased gradually in the human gastric cancer tissues, paracarcinoma tissues and normal tissues. The opinion that *BRMS1* could inhibit the activity of NF-κB to inhibit the metastasis of tumor was supported by many researches [3–5,15,16]. As Liu *et al.* [3] found that *BRMS1* could be co-immunoprecipitated with RelA/p65, the subunit of NF-κB. Findings of Cicek *et al.* [17] also showed that *BRMS1* could inhibit the phosphorylation of IκBα, as well as The NF-κB nuclear transportation and activation. Li *et al.* [15] held that NF-κB inhibitor could inhibit the invasion of gastric cancer cell and NF-κB signal transduction pathway, which could be used as the potential treatment strategy for the gastric cancer. This study also proved that the phosphorylation of NF-κB p65 and IκBα was reduced in *BRMS1* recombinant plasmid transfection group.

BRMS1 could inhibit the reconstruction of cytoskeleton and the formation of cell adhesion protein [18]. The osteopontin is some kind of secretory phosphorylated glycoprotein, which can be interacted with the extracellular matrix and then used as the signal molecule to be involved in the cell adhesion, migration, immune reaction and inflammatory reaction. *BRMS1* shows the negative correlation with the osteopontin in the hepatocellular carcinoma cells. Besides, *BRMS1* would not affect the growth of SK-Hep1 cells, while *BRMS1* transfection could significantly inhibit the expression of osteopontin [19]. The high metastasis of *BRMS1* contributed to the increased expression of osteopontin in the glioma cell lines [20]. The transfection of osteopontin RNAi in the gastric cancer cell

BGC-283 could significantly inhibit the growth, migration and invasion of gastric cancer cells and down-regulate the expression of MMP-2 and binding activity of NF-κB DNA. The *in vivo* animal experiments also proved that, compared with the wild mice, the osteopontin siRNA could significantly inhibit the growth of tumor and prolong the survival of mice [21]. The gene silencing of osteopontin could significantly inhibit the growth, invasion and metastasis of tumor in the transplanted tumor mice model with gastric cancer and also down-regulate the expression of MMP-2 and MMP-9 through The NF-κB signal pathway [22]. This study also proved the down-regulated expression of osteopontin in *BRMS1* recombinant plasmid transfection group, which indicated that *BRMS1* could regulate the expression of osteopontin through The NF-κB signal pathway and thus inhibit the adhesion and invasion of gastric cancer cells.

E-cadherin is the transmembrane protein on the cell surface, as some kind of epithelial cadherin, which can medicate the adhesion among same cells and between cells and play the key role in maintaining the cellular morphology, signal transduction and integrity of tissue structure. E-cadherin is regarded as the selective adhesive of epithelial cells and its down-regulated expression or missing will dissociate the epithelial cells and thus cause the dissociation of malignant tumor from the primary focus during the metastasis and result in the metastasis and spread of tumor cells. The missing or down-regulated expression of E-cadherin can promote the malignancy of gastric cancer and suggest a bad prognosis for patients with tumor [23]. The interference expression of E-cadherin in the ovarian cancer cell RMUG-S could significantly promote the adhesion and invasion of cancer cells [24]. When transplanting the E-cadherin gene in the tumor cells with the high level and stable expression, it could reduce or disappear the invasion of tumor cells [25]. In the gastric cancer cell SGC-7901, the expression of E-cadherin could be inhibited through The NF-κB signal pathway and thus the adhesion and metastasis of cancer cells could be improved [26]. This study also proved the up-regulated expression of E-cadherin in the *BRMS1* recombinant plasmid transfection group, which indicated that *BRMS1* could regulate the expression of E-cadherin through The NF-κB signal pathway and thus inhibit the adhesion and invasion of gastric cancer cells.

The degradation of extracellular matrix is the essential step for the invasion and metastasis of tumor. MMPs are the most important group of proteinases in the degradation of extracellular matrix, which plays the critical role in the invasion and metastasis of cell tumor and is also the key enzyme in these processes. MMP-9 is some kind of proteolytic enzyme secreted by various cells, as the enzyme with the largest molecular weight among MMPs, which can degrade the extracellular matrix and basement membrane, improve the movement of cells and promote the spread and metastasis of tumor. MMPs played the critical role in the invasion of hepatocellular carcinoma cells [27]. Yang *et al.* [28] found the significantly over-expression of MMP-9 in the gastric cancer tissue, while the expression of MMP-9 in patients with the distant metastasis was significantly higher than the one in patients without the distant metastasis. According to the clinical meta analysis, the high expression of MMP-9 could promote the invasion of gastric cancer tissues and then affect the prognosis of patients with the gastric cancer [29]. In pathological conditions, there was the positive correlation between the activation of NF-κB and the expression of MMP-2 and MMP-9, which could affect the growth, proliferation, invasion and metastasis of tumor [30,31]. MMP-2 could not only degrade the gelatin and type IV collagen in the extracellular matrix that contributed to the infiltration of tumor cells along the damaged basement membrane around, but also promote the invasion and metastasis of tumor through the new capillaries, as the indicator for the infiltration and invasion of malignant tumor [32]. Kwon *et al.* [33] proved that MMP-2 siRNA could inhibit the migration of gastric cancer cells and it was realized through The NF-κB signal pathway. This study also proved the down-regulated expression of MMP-2 and MMP-9 in *BRMS1* recombinant plasmid transfection group and indicated that *BRMS1* could regulate the expression of MMP-2 and MMP-9 through The NF-κB signal pathway and thus inhibit the invasion and metastasis of gastric cancer cells.

In conclusion, the expression of *BRMS1* does not affect the activity of mouse forestomach carcinoma or the size of transplanted tumor of nude mice. The increased expression of *BRMS1* can inhibit the adhesion, migration and invasion of gastric cancer cells. Such process is related to the down-regulated expression of osteopontin, MMP-2 and MMP-9 and the up-regulated expression of E-cadherin, which may be realized through The NF-κB signal pathway.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Cicek D, Kandi B, Oguz S, Ucak H, Saral Kurt A. The frequency and seasonal variations in juvenile outpatients treated in a dermatology polyclinic. *Int J Dermatol* 2008; **47**(11): 1121-1125.

[2] Samant RS, Seraj MJ, Saunders MM, Sakamaki TS, Shevde LA, Harms JF, et al. Analysis of mechanisms underlying BRMS1 suppression of metastasis. *Clin Exp Metastasis* 2000; **18**(8): 683-693.

[3] Liu Y, Mayo MW, Xiao A, Hall EH, Amin EB, Kadota K, et al. Loss of BRMS1 promotes a mesenchymal phenotype through NF-kappaB-dependent regulation of Twist1. *Mol Cell Biol* 2015; **35**(1): 303-317.

[4] Cicek M, Fukuyama R, Cicek MS, Sizemore S, Welch DR, Sizemore N, et al. BRMS1 contributes to the negative regulation of uPA gene expression through recruitment of HDAC1 to the NF-kappaB binding site of the uPA promoter. *Clin Exp Metastasis* 2009; **26**(3): 229-237.

[5] Samant RS, Clark DW, Fillmore RA, Cicek M, Metge BJ, Chandremouli KH, et al. Breast cancer metastasis suppressor 1 (BRMS1) inhibits osteopontin transcription by abrogating NF-kappaB activation. *Mol Cancer* 2007; **6**: 6.

[6] Sheng XJ, Zhou DM, Liu Q, Lou SY, Song QY, Zhou YQ, et al. BRMS1 inhibits expression of NF-kappaB subunit p65, uPA and OPN in ovarian cancer cells. *Eur J Gynaecol Oncol* 2014; **35**(3): 236-242.

[7] Mei P, Bai J, Shi M, Liu Q, Li Z, Fan Y, et al. BRMS1 suppresses glioma progression by regulating invasion, migration and adhesion of glioma cells. *PLoS One* 2014; **9**(5): e98544.

[8] Zhu Y, Liu Y, Qian Y, Dai X, Yang L, Chen J, et al. Research on the efficacy of *Celastrus orbiculatus* in suppressing TGF-beta1-induced epithelial-mesenchymal transition by inhibiting HSP27 and TNF-alpha-induced NF-kappa B/Snail signaling pathway in human gastric adenocarcinoma. *BMC Complement Altern Med* 2014; **14**: 433.

[9] Chen Z, Liu M, Liu X, Qiao Y. COX-2 regulates E-cadherin expression through the NF-kappaB/Snail signaling pathway in gastric cancer. *Int J Mol Med* 2013; **32**(1): 93-100.

[10] Peng H, Zhou W, Zhou H, Li Y, Zou X. Relationship between the expression of BRMS1 mRNA and the metastasis of lymph node with gastric carcinoma. *Chin J Curr Adv General Surg* 2007; **10**(6): 497-499.
[11] Shevde LA, Samant RS, Goldberg SF, Sikaneta T, Alessandrini A, Donahue HJ, et al. Suppression of human melanoma metastasis by the metastasis suppressor gene, BRMS1. *Exp Cell Res* 2002; **273**(2): 229-239.
[12] Zhang S, Lin QD, DI W. Suppression of human ovarian carcinoma metastasis by the metastasis-suppressor gene, BRMS1. *Int J Gynecol Cancer* 2006; **16**(2): 522-531.
[13] You J, He X, Ding H. BRMS1 regulates apoptosis in non-small cell lung cancer cells. *Cell Biochem Biophys* 2015; **71**(1): 465-472.
[14] Sheng Y, Long YM, Jian R, Xie WR. Nuclear factor kappa B: a marker of chemotherapy for human stage IV gastric carcinoma. *World J Gastroenterol* 2008; **14**(30): 4739-4744.
[15] Li WF, Hao DJ, Fan T, Huang HM, Yao H, Niu XF. Protective effect of chelerythrine against ethanol-induced gastric ulcer in mice. *Chem Biol Interact* 2014; **208**: 18-27.
[16] Miao L, Xiong X, Lin Y, Cheng Y, Lu J, Zhang J, et al. Down-regulation of FoxM1 leads to the inhibition of the epithelial-mesenchymal transition in gastric cancer cells. *Cancer Genet* 2014; **207**(3): 75-82.
[17] Cicek M, Fukuyama R, Welch DR, Sizemore N, Casey G. Breast cancer metastasis suppressor 1 inhibits gene expression by targeting nuclear factor-kappaB activity. *Cancer Res* 2005; **65**(9): 3586-3595.
[18] Khotskaya YB, Beck BH, Hurst DR, Han Z, Xia W, Huang M, et al. Expression of metastasis suppressor BRMS1 in breast cancer cells results in a marked delay in cellular adhesion to matrix. *Mol Carcinog* 2014; **53**(12): 1011-1026.
[19] Wu Y, Jiang W, Wang Y, Wu J, Saiyin H, Qiao X, et al. Breast cancer metastasis suppressor 1 regulates hepatocellular carcinoma cell apoptosis via suppressing osteopontin expression. *PLoS One* 2012; **7**(8): e42976.
[20] Metge BJ, Liu S, Riker AI, Fodstad O, Samant RS, Shevde LA. Elevated osteopontin levels in metastatic melanoma correlate with epigenetic silencing of breast cancer metastasis suppressor 1. *Oncology* 2010; **78**(1): 75-86.
[21] Gong M, Lu Z, Fang G, Bi J, Xue X. A small interfering RNA targeting osteopontin as gastric cancer therapeutics. *Cancer Lett* 2008; **272**(1): 148-159.
[22] Liu J, Liu Q, Wan Y, Zhao Z, Yu H, Luo H, et al. Osteopontin promotes the progression of gastric cancer through the NF-kappaB pathway regulated by the MAPK and PI3K. *Int J Oncol* 2014; **45**(1): 282-290.
[23] Liu X, Chu KM. E-cadherin and gastric cancer: cause, consequence, and applications. *Biomed Res Int* 2014; **2014**: 637308.
[24] Sawada K, Mitra AK, Radjabi AR, Bhaskar V, Kistner EO, Tretiakova M, et al. Loss of E-cadherin promotes ovarian cancer metastasis via alpha 5-integrin, which is a therapeutic target. *Cancer Res* 2008; **68**(7): 2329-2339.
[25] Chao YL, Shepard CR, Wells A. Breast carcinoma cells re-express E-cadherin during mesenchymal to epithelial reverting transition. *Mol Cancer* 2010; **9**: 179.
[26] Song W, Zhao C, Jiang R. Integrin-linked kinase silencing induces a S/G2/M phases cell cycle slowing and modulates metastasis-related genes in SGC7901 human gastric carcinoma cells. *Tumori* 2013; **99**(2): 249-256.
[27] Ordonez R, Carbajo-Pescador S, Prieto-Dominguez N, González-Gallego N, Mauriz J. Inhibition of matrix metalloproteinase-9 and nuclear factor kappa B contribute to melatonin prevention of motility and invasiveness in HepG2 liver cancer cells. *J Pineal Res* 2014; **56**(1): 20-30.
[28] Yang S, Zhao Z, Wu R. Expression and biological relationship of vascular endothelial growth factor-A and matrix metalloproteinase-9 in gastric carcinoma. *J Int Med Res* 2011; **39**(6): 2076-2085.
[29] Zhang QW, Liu L, Chen R, Wei YQ, Li P, Shi HS, et al. Matrix metalloproteinase-9 as a prognostic factor in gastric cancer: a meta-analysis. *Asian Pac J Cancer Prev* 2012; **13**(6): 2903-2908.
[30] Bi TQ, Che XM, Liao XH, Zhang DJ, Long HL, Li HJ, et al. Overexpression of Nampt in gastric cancer and chemopotentiating effects of the Nampt inhibitor FK866 in combination with fluorouracil. *Oncol Rep* 2011; **26**(5): 1251-1257.
[31] Tian C, Zhang L, Hu D, Zhang L, Hu D, Ji J. Ghrelin induces gastric cancer cell proliferation, migration, and invasion through GHS-R/NF-kappaB signaling pathway. *Mol Cell Biochem* 2013; **382**(1–2): 163-172.
[32] Chang A, Parikh P, Thongprasert S, Tan EH, Rerng RP, Ganzon D, et al. Gefitinib (IRESSA) in patients of Asian origin with refractory advanced non-small cell lung cancer: subset analysis from the ISEL study. *J Thorac Oncol* 2006; **1**(8): 847-855.
[33] Kwon CH, Moon HJ, Park HJ, Jin HC, Park DY. S100A8 and S100A9 promotes invasion and migration through p38 mitogen-activated protein kinase-dependent NF-kappaB activation in gastric cancer cells. *Mol Cells* 2013; **35**(3): 226-234.