Natural compound Byakangelicin suppresses breast tumor growth and motility by regulating SHP-1/JAK2/STAT3 signal pathway

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Research Article

Keywords: Byakangelicin, breast cancer, growth and motility, SHP1, JAK2/STAT3 signaling

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Natural compound Byakangelicin suppresses breast tumor growth and motility by regulating SHP-1/JAK2/STAT3 signal pathway

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Key Points

• Byakangelicin exerts anti-breast cancer effect in vitro.

• Byakangelicin inhibits growth and motility in breast cancer cells.

• Byakangelicin functions via blocking JAK2/STAT3 signal pathway in breast cancer cells.

• Byakangelicin impairs JAK2/STAT3 signal pathway via inducing SHP1.
Abstract

Introduction

Byakangelicin is one of the furanocoumarins extracted from the root of Byakangelicin and has protective effect on liver injury and fibrosis. In addition, Byakangelicin, as a traditional medicine, is also used to treat colds, headache and toothache. Recent studies have shown that Byakangelicin exhibits anti-tumor function; however, the role of Byakangelicin in breast tumor progression and related mechanism has not yet been elucidated. Our study aims to investigate the role of Byakangelicin in breast tumor progression and the underlying mechanism.

Methods

To measure the effect of Byakangelicin on JAK2/STAT3 signaling, a dual luciferase reporter assay and a western blot assay were performed. CCK8, colony formation, apoptosis and cell invasion assays were used to examine the inhibitory potential of Byakangelicin on breast cancer cells. Additionally, SHP-1 was silenced by specific siRNA duplex and the function of SHP-1 on Byakangelicin-mediated inhibition of JAK2/STAT3 signaling was evaluated.

Results

Byakangelicin treatment significantly inhibited STAT3 transcriptional activity. In addition, Byakangelicin treatment blocked JAK2/STAT3 signaling in a dose-dependent manner. Byakangelicin-treated tumor cells showed a dramatically reduced proliferation, colony formation and invasion ability. Moreover, Byakangelicin remarkably induced breast cancer cell apoptosis. Furthermore, Byakangelicin regulated the expression of SHP1.

Conclusion

In conclusion, our current study indicated that Byakangelicin, a natural compound, inhibits SHP-1/JAK2/STAT3 signaling and thus blocks tumor growth and motility.

Keywords

Byakangelicin, breast cancer, growth and motility, SHP1, JAK2/STAT3 signaling
1. Introduction

Breast cancer is one of the most widespread tumors in women. Although enhanced prediction and treatment strategies for breast cancer are available, more than 400,000 breast cancer-related deaths are reported in the US, mainly due to tumor metastasis and high recurrence rates\(^1\). Therefore, there is an urgent need to develop a novel therapeutic strategy for breast cancer treatment to improve the clinical outcomes.

Signal transducers and activators of transcription (STAT) family, which is contained seven members, namely, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6, is essential for tumor proliferation, differentiation, apoptosis, multidrug resistance and immune response\(^2\). The receptor-associated tyrosine kinases of the Janus-Kinase (JAK) family are named upstream of STATs, which comprise JAK1, JAK2, JAK3 and TYK2\(^3\). Once cytokines (such as IL6, EGF, etc) bind to their corresponding receptors, JAKs are activated and lead to the phosphorylation of a broad range of STAT proteins\(^4\); phosphorylated STATs then forms homo- or hetero-dimers and transfer to the nucleus. Nuclear STAT dimers recognize the specific motif in target gene promoters, thus regulating gene transcription\(^5\).

Among STAT family, STAT3 has been extensively studied in the field of oncology. Research reported activation of STAT3 is associated with a poor prognosis in a series of cancers, such as lung cancer\(^6\), gastric cancer\(^7\), osteosarcoma\(^8\) and so on. STAT3 promotes tumor growth and progression through regulation of the expression of downstream target genes, including proliferation related genes (Bcl-2, Survivin, Bcl-xL, Cyclin D1and Mcl-1), angiogenesis related genes (HIF-1alpha and VEGF) and etc\(^9,10\). STAT3 is activated in about 40 % of breast pathological specimen and there's a growing body of evidence that pharmacological inhibition STAT3 activation can significantly block breast cancer growth and progression\(^11\). Therefore, targeting STAT3 may be a potential therapeutic approach for breast cancer treatment. Plant-based natural drugs are an important source of novel drug development. There are several natural product STAT3 inhibitors that are currently in research for use as potential anti-cancer therapeutics for preclinical research. Examples are trienomycin A\(^12\), pectolinarigenin\(^13\),
and 10,11-dehydrocurvularin\textsuperscript{14}, which have shown significant efficacy in blocking STAT3 activation. Byakangelicin (Bya) is one of the furanocoumarins extracted from the root of Bya and exhibits protective effect on liver injury and fibrosis\textsuperscript{15}. Bya, as a traditional medicine, is also used to treat colds, headache and toothache\textsuperscript{15}. Recent studies have indicated that Bya shows anti-tumor function; however, the role of Bya in breast tumor progression and related mechanism has not yet been elucidated.

2. Materials and methods

2.1. Reagent

Bya was purchased from MCE (HY-N6022). Flag-M2 beads were obtained from Sigma. Dual-Luciferase Reporter Assay and CCK-8 kits were from Shanghai Yi Sheng Biotechnology. The Apoptosis Detection Kit and Matrigel were from BD Biosciences. Lipofectamine 3000 was purchased from Invitrogen. Antibodies recognizing p-STAT3 (Y705), STAT3, p-JAK2 (Y1007/1008), JAK2, Survivin, Bel-2, Mcl-1, VEGF, HA, PARP, N-cadherin, Vimintin, Snail, E-cadherin, SHP-1 and SHP-2 were purchased from Cell Signaling Technology Inc. Antibody against actin and Flag was purchased from Sigma-Aldrich.

2.2. Cell culture

Human breast cancer cell MDA-MB231, 4T1 and T47D were obtained from ATCC and cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) (GIBCO, 41965-039), supplemented with 10% FBS and 1% penicillin/streptomycin. Mouse breast cancer cell line 4T1 was cultured in RPMI1640 (Sigma-Aldrich, R8758), supplemented with 10% FBS and 1% penicillin/streptomycin. All cell lines were maintained in the incubator at 37°C, 5% CO\textsubscript{2}. All cell lines were tested and confirmed to be negative for mycoplasma infection.

2.3. Dual luciferase reporter assay

MDA-MB231 cells were transfected with STAT3 reporter plasmid (pGMSTAT3-Luc, Shanghai Yi Sheng Biotechnology Co. Ltd.) and pRL-SV40 (Renilla luciferase) using lipofectamine 3000 (Invitrogen) according to the manufacturer's guidelines. 24 h post transfection, cells were treated with different concentrations of Bya for 24 h. Luciferase
activity was determined by using the Dual-Luciferase Reporter Assay System (Shanghai Yi Sheng Biotechnology Co. Ltd.). Renilla luciferase activity was used as an internal control.

2.4. Western blot assay

Breast cancer cells were harvested with RIPA buffer supplemented with protease and phosphatase inhibitors. Protein concentrations were determined using the Bradford assay and samples were subjected to SDS-PAGE, transferred onto a PVDF membrane (Millipore), and incubated with indicated primary antibodies. After 3 washes with TBST, the membranes were incubated with HRP-conjugated secondary antibodies and visualized by ECL kits.

2.5. Transfection

Flag- and HA-tagged STAT3 vector were obtained from Changsha youbaoBiotechnology Co. Ltd. Flag- and HA-tagged STAT3 were transfected in MDA-MB231 cells using lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. For siRNAs-directed knockdown, MDA-MB231 cells were transfected 100nm siRNAs (siRNA1: 5’-GCAGGAGGUGAAACUUG-3’ and siRNA2: 5’-CCAGUUCAUUGAAACCAUTAA-3’) targeting SHP-1 using lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions.

2.6. Co-immunoprecipitation assay

MDA-MB231 cells transiently transfected with Flag- and HA-tagged STAT3 were treated with indicated concentrations of Bya and lysed with NP40 lysis buffer. Equal amount of cell lysates were incubated with Flag-M2 beads at 4°C overnight. Beads were then washed with lysis buffer and resuspended in loading buffer and boiled at 100°C for 10 min. Samples were separated on SDS-PAGE.

2.7. Immunofluorescence assay

MDA-MB231 cells were incubate in the presence or absence of Bya for 24 h and stimulated with IL-6. Cells were then washed with PBS, fixed with 4% PFA, permeabilized with 0.5% Triton X-100 and blocked with 1% BSA/PBS. Cells were incubated with indicated primary antibodies followed by Alexa Fluor 488 secondary antibody. Nucleus was counter stained with DAPI.
2.8. CCK-8 assay

Breast cancer cells were seeded in the 96-well plates and treated with increasing concentrations of Bya. After 48 h, CCK-8 solution was added (10uL/well) and the absorption was determined at 450 nm by a microplate spectrophotometer (Thermo). To examine the role of SHP-1 in Bya-mediated STAT3 inhibition, SHP-1 was knocked down in breast cancer cells and indicated concentration of Bya was added. 48 h post incubation, CCK-8 solution was added and the absorption at 450 nm was determined.

2.9. Colony formation assay

Single cell suspensions of T47D and 4T1 cells were seeded into 6-well plates to allow attachment. Cells were then treated with indicated concentrations of Bya for a week. Media were replaced every other day. When the assay was terminated, colonies were fixed with 4% PFA and stained with crystal violet.

2.10. Apoptosis assay

Apoptosis assay was performed as described previously\textsuperscript{16}. Cell apoptosis were determined using the Apoptosis Detection Kit (BD Biosciences) according to the manufacturer's instructions. Breast cancer cells were treated with Bya for 48 h, followed by annexin V–FITC and PI staining before evaluation by flow cytometry.

2.11. Invasion assay

Invasion assay was performed as described previously\textsuperscript{17}. Briefly, breast cancer cells were treated with Bya for 12 h. Single cell suspensions were added in the transwell upper chamber pre-coated with matrigel, and the complete medium was added in the bottom. Indicated concentration of Bya was added in both chambers. After 12 h, the invaded cells were fixed and stained with 0.1% crystal violet and counted manually. To evaluate the effect of SHP-1 on Bya-mediated invasion inhibition, breast cancer cells were transfected with siRNAs targeting SHP-1 for 24 h and treated with Bya for 12 h. Cell suspensions containing Bya were added in the transwell upper chamber pre-coated with matrigel, and complete medium with indicated Bya was added in the bottom.

2.12. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 software (GraphPad
Student’s t test was used for the comparison of measurable differences between two groups and was conducted on two samples from each experiment to determine the statistical significance of the differences. P value of <0.05 was considered statistically significant.

3 Results
3.1 Bya functions through inhibiting constitutive STAT3 signaling

The transcriptional factor STAT3 and its downstream signaling pathways are known to regulate multiple tumor-associated biological processes. To determine the role of Bya (Bya, MW:334.32, Figure 1A) in mediating STAT3 signaling, breast cancer cells were transfected with the STAT3 luciferase reporter plasmid and incubated with Bya. A dual luciferase assay was then performed to examine the STAT3 transcriptional activity. The results indicated treatment with Bya potently repressed STAT3 activity in a dose-dependent manner (Figure 1B). Tyrosine phosphorylation of STAT3 is crucial for its activation. We found that constitutively activated STAT3 in human and mouse breast cancer cells were largely inhibited in response to Bya exposure (Figure 1C). Several tyrosine kinases including the Janus kinase family are required for STAT3 phosphorylation and subsequent activation. Our results revealed that Bya repressed JAK2 phosphorylation, indicating the inhibitory effect of Bya on STAT3 may attribute to the upstream kinase repression. We next evaluated STAT3 downstream targets protein level after Bya treatment by immunoblotting. We found the expression of Bcl-2, Mcl-1, survivin, and VEGF was impaired upon Bya exposure (Figure 1D). STAT3 dimerization plays a causal role in its nuclear translocation and activation. We therefore expressed Flag-tagged and HA-tagged STAT3 in breast cancer cells and treated them with Bya. Our co-immunoprecipitation analysis showed Flag-tagged STAT3 interacted with its HA-tagged counterpart, indicating the constitutive STAT3 activation. However, the interplay was inhibited by Bya. Together, our results suggest that Byainhibited constitutive STAT3 signaling.
3.2 Bya inhibits IL-6 induced STAT3 activation and nuclear redistribution

We have demonstrated Byablocked STAT3 tyrosine phosphorylation at Y705 and its downstream targets expression. We thus hypothesized that STAT3 nuclear redistribution may be impaired by Bya as tyrosine phosphorylation of STAT3 is essential for its nuclear translocation and the subsequent activation. Like the other cytokines, IL-6 activates tyrosine kinases such as Janus-like kinases (JAKs) and c-Src to elicit STAT3 signaling. Our immunoblotting analysis indicated addition of IL-6 induced STAT3 Y705 phosphorylation in breast cancer cells, which was largely reduced byBya treatment (Figure 2A). Consistently, STAT3 translocated into the nucleus in response to IL-6 and administration of Bya blocked it (Figure 2B).Our data indicate that Bya exhibited the ability to repress the inducible STAT3 activation.

3.3 Bya effectively impairs breast cancer cell growth and motility

In an attempt to identify the inhibitory effects of Bya on breast cancer cells, we treated human MDA-MB-231, T47D cells and mouse 4T1 with indicated concentrations of Bya. Administration of Bya potently suppressed breast cancer cell viability (Figure 3A). OF note, Bya significantly reduced the clonogenicity of breast cancer cells (Figure 3B). We next sought to explore whether the cytotoxicity of Bya in breast cancer cells were ascribed to the increased apoptosis. Flow cytometry analyses revealed that Byainduced breast cancer cell apoptosis in a dose-dependent manner, which was in consistent with increased cleaved PARP (Figure 3C). In addition, we pre-coated the transwell inserts with Matrigel and found addition of Byablocked breast cancer cell invasion through the Matrigel (Figure 3D). Epithelial-mesenchymal transition (EMT) is a well-established to promote cancer cell invasion capability. We found Bya treatment increased epithelial marker E-cadherin expression and reduced mesenchymal molecules such as N-cadherin, vimentin, and snail (Figure 3E). These results indicate that Bya exhibits anti-tumor activity on breast cancer cells.

3.4 Bya upregulated the expression of SHP-1

SHP-1 acts as a non-transmembrane phosphatase to inhibit the activation of JAK family tyrosine kinases via its SH2 domains, and SHP-1 may be considered as a negative regulator in JAK/STAT3 signal pathway. We treated breast cancer cells
with a tyrosine phosphatase inhibitor (sodium vanadate) and Bya. We found Bya caused a decrease in STAT3 Y705 phosphorylation, which was rescued by sodium vanadate (Figure 4A), indicating the involvement of tyrosine phosphatase in Bya-mediated STAT3 inhibition. In parallel, the protein level of SHP-1 increased when breast cancer cells were incubated with Bya (Figure 4B). To explore the role of SHP-1 in Bya-mediated STAT3 inhibition, we knocked down SHP-1 with specific siRNA (Figure 4C), and the CCK8 assays demonstrated that the inhibitory effect of Bya on cell viability was partially impaired by SHP-1 silence (Figure 4D). Furthermore, Bya possessed the ability to inhibit breast cancer cell invasion; however, this effect was reversed by knocking down of SHP-1 (Figure 4E). Our results indicate that Bya repressed STAT3 in a SHP-1-dependent manner.

4. Discussion

Although Bya have been reported to display anticancer activities, its underlying molecular mechanism remains unclear. In this study, we utilized in vitro cell models to investigate the effect of Bya against breast cancer growth and motility. We found that Bya significantly inhibited breast cancer growth and motility by blocking the JAK2/STAT3 signaling. Importantly, we also found Bya markedly upregulated the expression of SHP1. This study could help to understand the pharmacology of Bya in the treatment of breast cancer.

Previous studies reported that Bya possess antitumor activity19. However, whether Bya could impair breast tumor progression is poorly understood. Our study first revealed that Bya significantly suppressed breast tumor cell proliferation in a dose-dependent manner and induced cell apoptosis. Successful invasion of tumor cells is necessary for tumor cells. We found Bya remarkably inhibited tumor cells invasion. These results implied Bya acts as an inhibitor of breast cancer growth and metastasis.

Activated STAT3, which promotes cell growth and metastasis, has been considered as an oncogene20. A number of natural compounds have been reported to inhibit STAT3 and display strong anticancer activity in breast cancer. For example, 3-Deoxy-2β,16-dihydroxynagilactone E, a natural compound from Podocarpus nagi, inhibited breast...
cancer cell growth and induced apoptosis via inhibiting JAK2/STAT3 signal pathway\textsuperscript{21}. In this study, we demonstrated that Bya inhibits constitutive and inducible STAT3 activation in breast cancer cells. A series of target of STAT3 signaling have been discovered, including pro-survival and anti-apoptotic related proteins. In our present study, Bya exhibited inhibitory effect on STAT3 downstream targets expression. The epithelial to mesenchymal transition (EMT) is essential for metastatic spread of breast cancer cells\textsuperscript{22,23}. As an important upstream regulator of EMT, STAT3 is able to induce EMT-mediated tumor metastasis\textsuperscript{24}. Here, we found Bya remarkably induced epithelial marker (E-cadherin) and reduced mesenchymal markers (N-cadherin, Vimentin, and Snail) expression. These results indicated that Bya may act as a STAT3 inhibitor.

SHP-1 and SHP-2 are Src homology 2 domain-containing tyrosine phosphatases with major pathological implications in tumor cells growth and metastasis regulating signaling\textsuperscript{25}. It has been proven that protein tyrosine phosphatases such as SHP1 and SHP2 are essentially involved in JAK2/STAT3 regulation\textsuperscript{9,26}. Sodium vanadate, a broad-acting tyrosine phosphatase inhibitor, could notably reverse Bya induced STAT3 inhibition without affecting the basal level of STAT3 phosphorylation, suggesting the involvement of tyrosine phosphatases in the process of Bya mediated STAT3 inhibition (Figure 4a). Here, we found Bya specifically induced SHP-1 expression, which implied SHP1 is essential for Bya-mediated JAK2/STAT3 signaling inhibition. However, the mechanism that Bya upregulates SHP1 still needs further research. We will further examine whether Bya regulates SHP1 via mRNA or protein level.

In conclusion, our current preclinical study demonstrated that Bya, a natural compound, inhibits SHP-1/JAK2/STAT3 signaling and thus blocking tumor growth and motility. We therefore conclude that the natural compound Bya may be a good potential candidate for treatment of breast cancer.

**Declarations**

**Conflict of interest** Xiuzhen Shi, Yuexing Lai, Wenjing Liu, Xi Zhang and Yanqin Cang declare that they have no conflicts of interest to declare.
Funding This work was sponsored by the Youth Project of Shanghai Municipal Health Commission(20194Y0462).

Availability of data and material Not applicable.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Code availability Not applicable.

Author contributions Yanqin Cang is the corresponding author. Yanqin Cang and Xiuzhen Shi designed the content and wrote the first draft; Yanqin Cang and Yuexing Lai produced the figures; Wenjing Liu and Xi Zhang produced the tables; and Xiuzhen Shi, Yuexing Lai and Yanqin Cang revised the manuscript. Yanqin Cang was responsible for supervision of the entire project.

References
7. Ashrafizadeh, M., et al. STAT3 Pathway in Gastric Cancer: Signaling, Ther


Figure legends

Fig1 Bya inhibits STAT3 activity. A, The chemical structure of Bya was shown. B, MDA-MB231 breast cancer cells were transfected with STAT3 luciferase reporter gene plasmid and treated with indicated concentrations of Bya for 24 h. The luciferase activity was measured. C, Breast cancer cells were left untreated or treated with Bya for 24 h. Cells were lysed and the samples were subjected to immunoblotting with indicated antibodies. D, MDA-MB231 cells were transfected with Flag- and HA-tagged STAT3, following a 24 h treatment with Bya. Immunoprecipitation analysis was performed with indicated antibodies. E, Breast cancer cells were treated with or without Bya for 24 h. The protein level of STAT3 downstream targets were detected by immunoblotting. ***P < 0.001 versus the control group.

Fig2 Bya exhibits the inhibitory effect on IL-6-induced STAT3 activation. A, MDA-MB231 cells were treated with Bya for 24 h, followed by IL-6 (20 ng/μl) stimulation for 30 min. The protein level of STAT3 Tyr705 was detected by immunoblotting. B, MDA-MB231 cells were treated with Bya for 24 h, followed by IL-6 stimulation. Immunofluorescence analysis was performed with anti-STAT3 antibody. Nucleus was counterstained with DAPI. Scale bar: 50 μm.
Fig 3 Bya impaired breast cancer cell growth and invasion. A, Breast cancer cells were incubated with indicated concentrations of Bya for 48 h. A CCK8 assay was performed. B, T47D and 4T1 cells were seeded in 6-well plates and treated with Bya for 7 days. The colonies were stained with 0.1% crystal violet. C, MDA-MB231 cells were treated with Bya for 48 h. Apoptotic cells were analyzed by flow cytometry (left) and immunoblotting (right). D, Single cell suspensions of MDA-MB231 and 4T1 were added in the transwell upper chamber precoated with Matrigel under indicated treatment. After 12 h, the invaded cells were fixed and stained with 0.1% crystal violet. Representative imaged were acquired. The invaded cells were quantified manually. E, MDA-MB231 and 4T1 cells were treated indicated dose of Bya for 24 h. Cell lysates were subjected to immunoblotting. ***P < 0.01 and ***P < 0.001 versus the control group.

Fig4 SHP-1 is crucial for Bya in the repression of breast cancer growth and invasion. A, MDA-MB231 and 4T1 cells were treated with vanadate and Bya for 24 h. STAT3 Tyr705 was examined by immunoblotting. B, MDA-MB231 and 4T1 cells were exposed to increasing concentrations of Bya for 24 h. The protein level of SHP-1 was detected. C, MDA-MB231 cells were transfected with siRNAs targeting SHP-1, and the knockdown efficiency was detected by immunoblotting. D, MDA-MB231 cells were transfected with siRNAs targeting SHP-1 followed by Bya treatment for 48 h. A CCK8 assay was performed. E, MDA-MB231 cells transfected with siRNAs targeting SHP-1 were added in the transwell upper chamber precoated with Matrigel under indicated treatment. 12 h post incubation, the invaded cells were fixed and stained with 0.1% crystal violet. The invaded cells were quantified manually. ***P < 0.001 versus the control group.
A

Byakangelicin
C_{17}H_{18}O_{7} FW: 334.32

B

Luciferase STAT3 activity (% of control)

0    5   10   20    Bya (μM)

0   5   10   20

C

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D

Flag-STAT3 - + + +
HA-STAT3    + + + +

Bya 0 0 10 20 (μM)

IP:Flag

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![Images of Western blots and immunofluorescence](image)
Fig 3

A

Bya (μM) 0 2 5 10 20

Cell viability (% of control)

MDA-MB231

4T1

T47D

Bya (μM) 0 2 5 10 20

Cell viability (% of control)

Bya (μM) 0 2 5 10 20

Cell viability (% of control)

B

0 2 5 10 Bya (μM)

4T1

T47D

Bya (μM) 0 2 5 10

Invaded cells number (% of control)

Bya (μM) 0 2 5 10

Invaded cells number (% of control)

C

Bya (μM) 0 5 10

Annexin V

Bya (μM) 0 5 10

Annexin V

Bya (μM) 0 5 10

Annexin V

Bya (μM) 0 5 10

Annexin V

D

Bya (μM) 0 5 10 20

Invaded cells number (% of control)

Bya (μM) 0 5 10 20

Invaded cells number (% of control)

Bya (μM) 0 5 10 20

Invaded cells number (% of control)

Bya (μM) 0 5 10 20

Invaded cells number (% of control)

E

MDA-MB231 4T1

Bya (μM) 0 5 10 20

E cadherin

N cadherin

Vimentin

Snail

β-actin

PARP

Cleaved PARP

β-actin

Annexin V

PI

Colonies (% of control)

Colonies (% of control)

Colonies (% of control)

Colonies (% of control)
Fig 4

A  

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C  

Scrambled  
siRNA SHP1  
siRNA-1 SHP1  
siRNA-2 SHP1  

SHP1  

β-actin  

D  

Cell viability (% of control)  

Bya (μM)  

0  0  10  10  

siSHP-1  

- + - +  

***  

E  

siSHP1  

Bya  

- + - ++  

Invaded cells number  

Bya (μM)  

0  0  10  10  

siSHP-1  

- + - +  

***
Fig 5

IL6, EGF, etc.

JAK2

STAT3

Byakangelicin

SHP1

Bcl2, VEGF, Survivin, Mcl1, etc

Tumor growth and metastasis