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Soyasaponin Ag inhibits triple-negative breast cancer progression *via* targeting the DUSP6/MAPK signaling

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Abstract

Introduction. Soyasaponins are triterpenoid glycosides discovered in soybean and have anti-cancer properties. Soyasaponin A was reported to repress estrogen-insensitive breast cancer cell proliferation. This study intends to explore the role of one isomer of soyasaponin A, *i.e.* soyasaponin Ag (Ssa Ag), in triple-negative breast cancer (TNBC) development.

Material and methods. Bioinformatic databases were used to predict DUSP6 expression in breast cancer (BC) as well as the correlation between the expression of DUSP6 (or MAPK1, MAPK14) with the prognosis of patients with BC. The expression of DUSP6/MAPK signaling-related genes (DUSP6, MAPK1, and MAPK14) in TNBC cell lines was assessed *via* Western blot analysis and RT-qPCR. Levels of cell apoptosis proteins (Bax and Bcl-2) in TNBC cells were assessed *via* Western blot analysis. CCK-8 assay, colony formation assay, and flow cytometry analysis were conducted for the measurement of TNBC cell growth and apoptosis. *In vivo* xenograft assay was employed for investigating the biological influence of Ssa Ag on tumor growth.

Results. The poor prognosis of BC patients was linked to the aberrant expression of DUSP6/MAPK pathway genes. Low expression of DUSP6 or high expression of MAPK1 (or MAPK14) was correlated to poor prognosis. DUSP6 was downregulated while MAPK1 and MAPK14 were upregulated in TNBC cells versus normal cells. Ssa Ag upregulated DUSP6 expression while downregulated MAPK1 and MAPK14 expression, inhibiting the MAPK signaling pathway. Additionally, Ssa Ag promoted *in vitro* TNBC cell apoptosis and restrained cell growth, and repressed *in vivo* tumor growth.

Conclusions. Ssa Ag inhibited TNBC progression *via* upregulating DUSP6 and inactivating the MAPK signaling pathway. *(Folia Histochemica et Cytobiologica 2021, Vol. 59, No. 4, 291–301)*

Key words: soyasaponin Ag; DUSP6/MAPK signaling; triple-negative breast cancer; proliferation; apoptosis

Introduction

As the second leading cause of mortality induced by cancer in females, breast cancer (BC) accounts for nearly a quarter of all cancers [1–3]. Triple-negative breast cancer (TNBC) which comprises about one-fifth of all BCs, is a unique subtype of BC with early recurrence, poor prognosis, and low survival rates compared with other types of BC [4, 5]. TNBC is characterized by lacking expression of human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), and progesterone receptor (PR) [6, 7]. Increasing evidence suggests that factors of diet, weight and physical activity are closely associated with

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a high risk of TNBC [8]. To date, surgery accompanied with chemotherapy and radiotherapy has been widely applied for TNBC treatment, but the problem of side effects on adjacent normal tissues is still unsolved [9]. Thus, it is urgent to elucidate the molecular mechanism underlying TNBC for improving clinical outcomes in TNBC patients.

Soyasaponins are biologically active secondary plant metabolites that can be discovered in soybeans and other legumes [10]. Based on the aglycone (soyasapogenol) structure, soyasaponins are mainly divided into two groups as A and B [11]. Soyasaponin A contains sugar moieties at the C-3 and C-22 positions of the ring structures [12]. Soyasaponin A has eight isomers, named as Aa-Ah [13]. Soyasaponins have numerous biological properties, including anti-carcinogenic, anti-obesity, anti-inflammatory, and hepatoprotective effects [14–16]. Studies have demonstrated that soyasaponins are involved in tumor growth and cancer cellular processes, including in BC. For example, soyasapogenin A inhibits the proliferation of estrogen-insensitive human BC cell line, MDA-MB-231 [17]. Soyasaponin I depresses cellular a2,3-sialyltransferase (ST) activity, thereby enhancing BC MCF-7 cell adhesion to the Matrigel-matrix and attenuating BC MDA-MB-231 cell migration [18]. Soyasapogenin A results in potent cytotoxicity to BC MDA-MB-231 cells and induces cell cycle arrest [19]. Nevertheless, the biological function and regulatory mechanism of soyasaponin Ag (Ssa Ag) in TNBC have not been investigated.

Mitogen-activated protein kinase (MAPK) participates in many signaling pathways that are crucial to regulating several cellular processes [20]. The MAPK pathway is closely related to the tumorigenesis of numerous cancers. For example, the activation of the ERK/MAPK pathway exerts promoting function on colorectal cancer [21]. MAPK/ERK-BIM signaling pathway is reported to be inhibited by SOX7 to attenuate cell apoptosis in lung cancer [22]. Dual specificity phosphatase 6 (DUSP6), or MAPK phosphatase 3 (MKP3), encodes proteins that belong to the bispecific specificity protein phosphatase subfamily [23, 24]. These phosphatases play biological roles in the negative regulation of the MAPK superfamily, including JNK, MAPK, and p38, and are closely related to cell proliferation and motion [25–27]. Emerging evidence has reported that DUSP6 exerts inhibitory function on malignant phenotypes of various cancers [28–32]. Previously, soyasapogenol I-αa was reported to exert anti-inflammatory effects via inhibiting the MAPK signaling pathway [33], revealing the role of soyasaponin in mediating the MAPK pathway. Nevertheless, whether Ssa Ag regulates the expression of DUSP6 and the MAPK pathway in TNBC remains unexplored.

Our study is intended to figure out the biological function of Ssa Ag in TNBC and explore the influence of Ssa Ag on the DUSP6/MAPK pathway. Our findings may provide an innovative strategy for TNBC treatment.

Materials and methods

Cell culture. Validated TNBC cell lines, MDA-MB-468 and MDA-MB-231 were identified for the study according to previous studies [34–36]. Human normal breast epithelial cell line MCF-10A and two TNBC cell lines were obtained from ATCC (Manassas, VA, USA). All cells were incubated in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) that contains 10% fetal bovine serum (Hyclone, USA), 100 μg/mL streptomycin, and 100 U/mL penicillium at 37°C with 5% CO_2. Ssa Ag was isolated and purified as previously described [13, 37]. TNBC cells were treated with Ssa Ag (1, 2, 4 μM) for 24 h to investigate the influence of soyasaponin A on cell malignant behaviors [38]. The concentration of DMSO as diluent control was taken as < 0.1%.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from cells applying TRIzol reagent (Invitrogen). Total RNA was reverse transcribed into cDNA with the PrimeScript RT Reagent kit (TaKaRa, Dalian, China). Then quantitative PCR was conducted using SYBR Premix Ex Taq (TaKaRa) to determine DUSP6, MAPK1, and MAPK14 expression. GAPDH served as the normalization control. The $2^{-\Delta\Delta Ct}$ method was used for analyzing the data.

Western blot analysis. Cell lysates were harvested by RIPA buffer (Invitrogen). Next, 20 μg of protein sample was transferred onto a PVDF membrane after being isolated by 10% SDS-PAGE. The membrane was blocked with 5% non-fat milk powder, and incubated with the primary antibodies (Abcam, Shanghai, China) as follows: anti-DUSP6 (ab76310, 1:500), anti-p38 alpha/MAPK14 (ab170099, 1:1000), anti-MAPK1 (ab32081, 1:1000), anti-Ki67 (ab92742, 1:5000), anti-CDK2 (ab32147, 1:1000), anti-Bax (ab32503, 1:1000), anti-Bcl-2 (ab182858, 1:2000) and anti-GAPDH (ab9485, 1:2500) overnight at 4°C. After washing with PBS at room temperature thrice (5 min each time), the membrane was incubated with a secondary antibody at 37°C for 1 h. The membrane was then rinsed thrice with PBS, immersed in a reinforced chemiluminescence reaction solution for 1 min, and quantified using Image software.

Cell Counting Kit-8 (CCK-8) assay. After treatment with Ssa Ag (1, 2, and 4 μM), TNBC cells were plated into 96-well plates (1×10^4 cells/well). The CCK-8 (Dojindo Laboratories, Kumamoto, Japan) reagent was added 24 h after different

treatments. Subsequently, cells were incubated for 2 h at 37°C, and a microplate reader was applied to evaluate the absorbance at 450 nm.

Colony formation assay. TNBC cells after Ssa Ag treatment were cultured in the medium plate for 14 days in a humidified ambiance with 5% CO_2 at 37°C. Afterward, cells were washed twice with PBS, fixed with 5% paraformaldehyde for 30 min, and stained with 1 mL of 0.1% crystal violet solution. At last, the cells were rinsed with PBS and the colony numbers were counted using a microscope (KEYENCE, Osaka, Japan).

Caspase-3 activity analysis. A Caspase-3 Colorimetric Assay Kit (R&D Systems, Minneapolis, MN, USA) was used to determine Caspase-3 activity. TNBC cells were lysed and centrifuged to acquire supernatants. The Bradford Protein Assay Kit (Beyotime) was employed to quantify the protein concentrations of each sample. The supernatants were mixed with buffer containing the substrate peptides for caspase-3 attached to p-nitroanilide (pNA) and incubated at 37°C for 2 h. The microplate reader was applied to measure absorbance at 405 nm.

Flow cytometry analysis. Cell apoptosis was detected by Annexin V-FITC/PI staining using flow cytometry. Cells were collected and rinsed with precooled PBS thrice after 24 h of Ssa Ag treatment. Then, cells (1×10^5 cells/mL) were stained with a binding buffer containing Annexin V-FITC and propidine iodide (PI) under darkness for 15 min at 4°C. Finally, cell apoptosis was evaluated with flow cytometry (BD Biosciences).

***In vivo* xenograft assay.** Athymic BALB/c nude female mice (4 weeks old; 18–20 g) bought from NihonClea, Japan was orientated for 14 days. Then, animals were pre-fed with vehicle (0.1% carboxymethyl cellulose (CMC)) or Ssa Ag supplemented vehicle (15 mg/kg) in 250 μL suspension twice a week for two weeks. MDA-MB-231 cells (5×10^6 in 200 μL PBS) were subcutaneously injected over the left and right thigh flanks. Seven days after the injection, mice were regularly fed for 28 days. Tumors were regularly monitored and sized with a Vernier caliper. Before tumors grew to approximately 1.5 cm in length, mice were sacrificed and the tumors in internal organs were examined.

Statistical analyses. Experimental assays were conducted in triplicate and the data are presented as the mean ± standard deviation. Comparisons were made by Student's *t*-test or one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* analysis. All statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL, USA), and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). The value of $p < 0.05$ was assumed to be statistically significant.

Results

DUSP6/MAPK pathway-related genes are correlated with poor prognosis of BC patients

The GEPIA database shows that DUSP6 expression at the mRNA level is significantly downregulated in 1085 BRCA tissue samples *vs.* that in 291 normal tissue samples (Fig. 1A). The Kaplan-Meier plotter database displays that low DUSP6 mRNA expression is correlated to the poor prognosis of BC patients (Fig. 1B). igh mRNA levels of MAPK1 and MAPK14 genes bring unfavorable prognosis of BC patients (Fig. 1C, D). According to these data, we assumed that the DUSP6/MAPK pathway is associated with BC development. Subsequently, the expression of DUSP6/MAPK pathway-related genes in TNBC cells *versus* normal cells was detected. Both the mRNA and protein levels of DUSP6 were downregulated while those of MAPK1 and MAPK14 were upregulated in TNBC cells *versus* normal cells (Fig. 1E, F). These data suggest that the DUSP6/MAPK pathway participates in mediating BC development.

Ssa Ag upregulates DUSP6 expression and inactivates the MAPK signaling pathway

The chemical structure of Ssa Ag was displayed in Fig. 2A. As shown by Western blot, DUSP6 protein level was markedly elevated in TNBC cells treated with Ssa Ag (1–4 μM) while MAPK1 and MAPK14 protein levels were markedly reduced by increasing concentration of Ssa Ag (Fig. 2B), indicating that Ssa Ag upregulates DUSP6 expression and inhibits the MAPK pathway.

Ssa Ag suppresses cell proliferation

To explore the biological role of Ssa Ag in TNBC, CCK-8 assays were conducted, which demonstrated that the viability of TNBC cells treated with Ssa Ag (1–4 μM) was significantly decreased *vs.* the control group (Fig. 3A). Colony formation assay indicated that colony numbers were markedly reduced by Ssa Ag versus the control and DMSO group (Fig. 3B,C). Additionally, the protein levels of CDK2 and Ki67 that are related to cell proliferation were decreased in TNBC cells treated with Ssa Ag (2 μM and 4 μM), as shown by Western blot (Fig. 3D). The findings above demonstrate that Ssa Ag suppresses the proliferation of TNBC cells.

Ssa Ag facilitates cell apoptosis

Flow cytometry analysis revealed that the cell apoptosis rate in TNBC cells treated with Ssa Ag (2–4 μM) were significantly increased *vs.* the control group (Fig. 4A). Additionally, caspase-3 activity analysis showed that the activity of caspase-3 in TNBC cells treated with

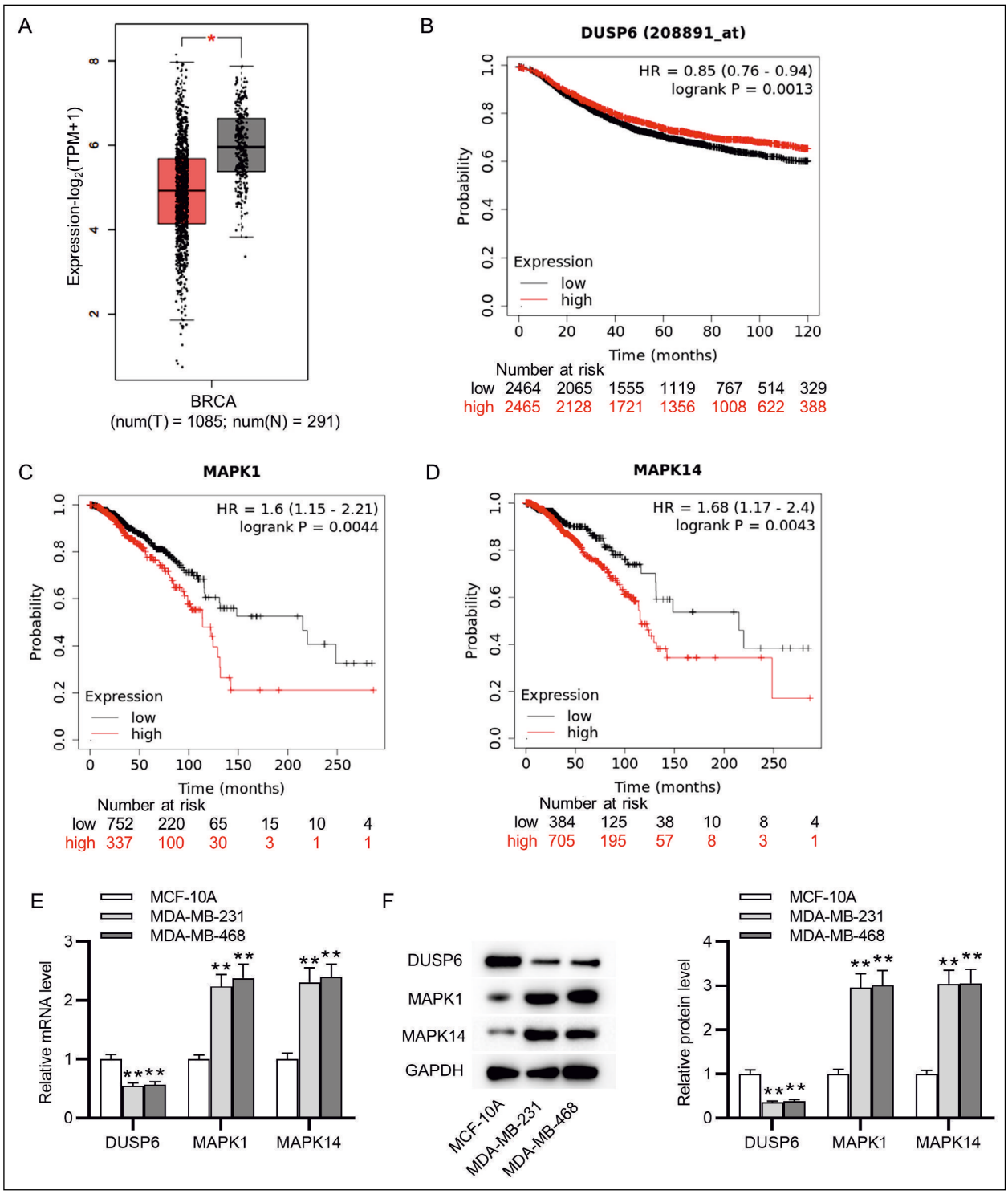

Figure 1. The expression of DUSP6/MAPK pathway-related genes in TNBC cells. **A.** DUSP6 expression at the mRNA level in 1085 breast cancer (BRCA) tissues and 291 normal tissues was shown in the GEPIA database (http://gepia.cancer-pku.cn/). **B–D.** The correlation between the mRNA levels of DUSP6, MAPK1, and MAPK14 genes with the prognosis of patients with BC was shown in the Kaplan-Meier plotter database (https://kmplot.com/). **E–F.** DUSP6, MAPK1, and MAPK14 expression in normal MCF-10A cells *versus* TNBC cells were measured by RT-qPCR and Western blot, respectively. $^{*}p < 0.05$, $^{**}p < 0.01$.

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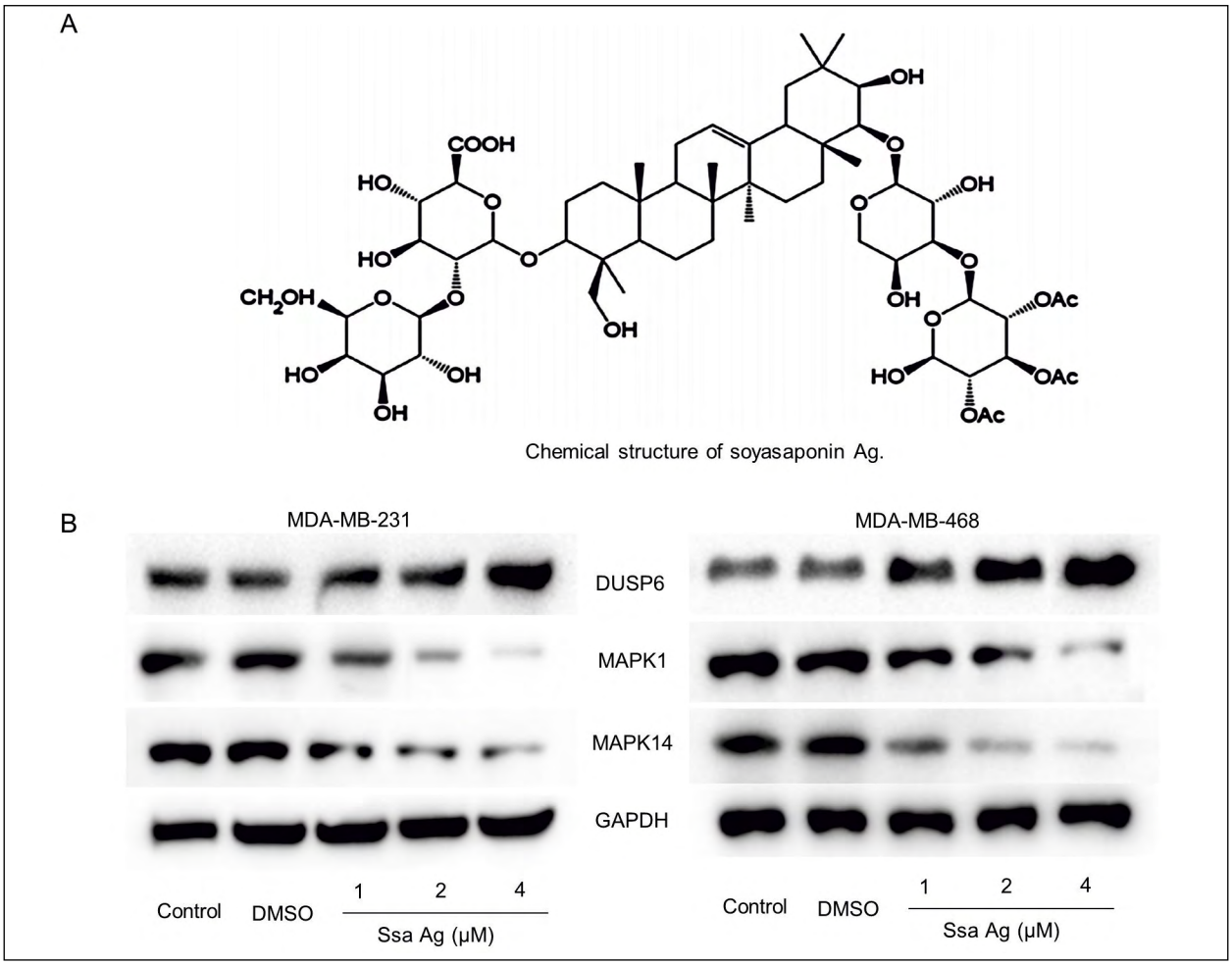

Figure 2. Ssa Ag upregulates the DUSP6 level and inactivates the MAPK signaling pathway. **A.** The chemical structure of Ssa Ag was shown. **B.** TNBC cells were treated with Ssa Ag (1, 2, 4 μM) for 24 h. Western blot was performed to measure DUSP6, MAPK1, and MAPK14 protein levels in TNBC cells.

Ssa Ag were markedly increased (Fig. 4B). The protein level of cell apoptosis marker Bax in TNBC cells was increased by Ssa Ag treatment while that of Bcl-2 was decreased by Ssa Ag (Fig. 4C). In summary, these results suggest that Ssa Ag promotes cell apoptosis in TNBC.

Ssa Ag suppresses tumor growth in TNBC

The effects of Ssa Ag on *in vivo* tumor growth were then examined. Mice were fed with Ssa Ag (15 mg/kg) twice a week before xenografting of MDA-MB-231 cells by subcutaneous injections. One week after injection, mice were fed with Ssa Ag (15 mg/kg) for the next 4 weeks before sacrifice (Fig. 5A) [38]. We discovered that *vs.* the vehicle group, the size of tumors treated with Ssa Ag was significantly decreased (Fig. 5B), and tumor in the Ssa Ag group grew at a slower speed than the control group (Fig. 5C). In addition, the tumor weight was markedly decreased by Snot-Ag versus the control group (Fig. 5D). The DUSP6 protein level in the tumors was increased by Ssa Ag while the levels of MAPK1 and MAPK14 were significantly decreased by Ssa Ag (Fig. 5E). In conclusion, Ssa Ag exerts a suppressive effect on tumor growth in TNBC.

Discussion

Saponins are bioactive molecules widely distributed in plants with rich structural and functional diversity. As such, they may be important substances worth investigating for potential drug applications [39, 40]. Previous studies have reported that soyasaponin exerts inhibitory function on malignant phenotypes of cancers [38, 41–45]. In detail, soyasaponin-I markedly suppressed the migratory ability of BC cells and participate in tumor cell invasion [43]. Soyasaponin Bb exerts promoting effect on cell apoptosis in esophageal cancer [45]. In this study, we discovered that Ssa Ag repressed the viability and proliferation of TNBC cells and facilitated TNBC cell apoptosis, yielding

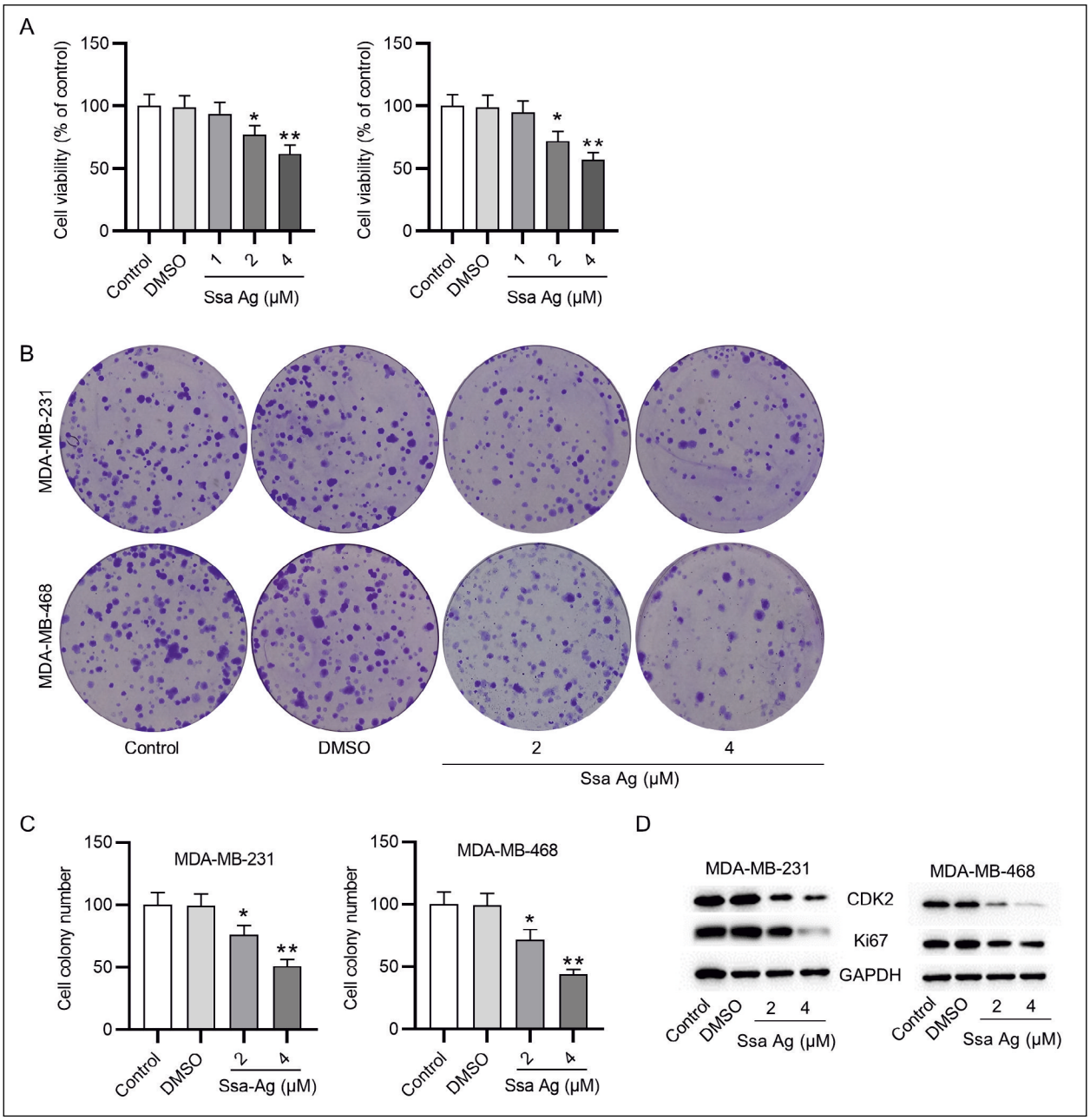

Figure 3. Ssa Ag inhibits cell proliferation in TNBC. **A.** TNBC cells were treated with Ssa Ag (1, 2, 4 μM) for 24 h. Cell viability was tested by CCK-8 assays. **B–C.** TNBC cells were treated with Ssa Ag (2 and 4 μM) for 24 h. The colony formation capacity of cells was evaluated *via* colony formation assays. **D.** TNBC cells were treated with Ssa Ag (2 and 4 μM) for 24 h. The protein levels of proliferation markers, CDK2 and Ki67, in TNBC cells were determined through Western blot. $^{*}p < 0.05$, $^{**}p < 0.01$.

similar results with previous studies. In addition, we conducted *in vivo* assays and Ssa Ag was found to be effective in inhibiting the volume and weight of tumors, further demonstrating that Ssa Ag plays a suppressive role in TNBC progression.

ERK signaling, a molecular switch involved in MAPK signaling, can be negatively regulated by DUSP6 [28]. Emerging studies have reported that DUSP6/MAPK signaling participates in the malignant progression of many diseases [46–51]. The activation of the MAPK pathway was also reported to be associated with the tumorigenesis of TNBC. For example, APP overexpression increases the phosphorylation levels of MAPK pathway components, including JNK3, MEK4, and MLK3, and the activation of the MAPK pathway promotes the EMT, migratory and invasive capabilities of MDA-MB-231 cells [52]. PRPF4 facilitates the growth, invasion, migration,

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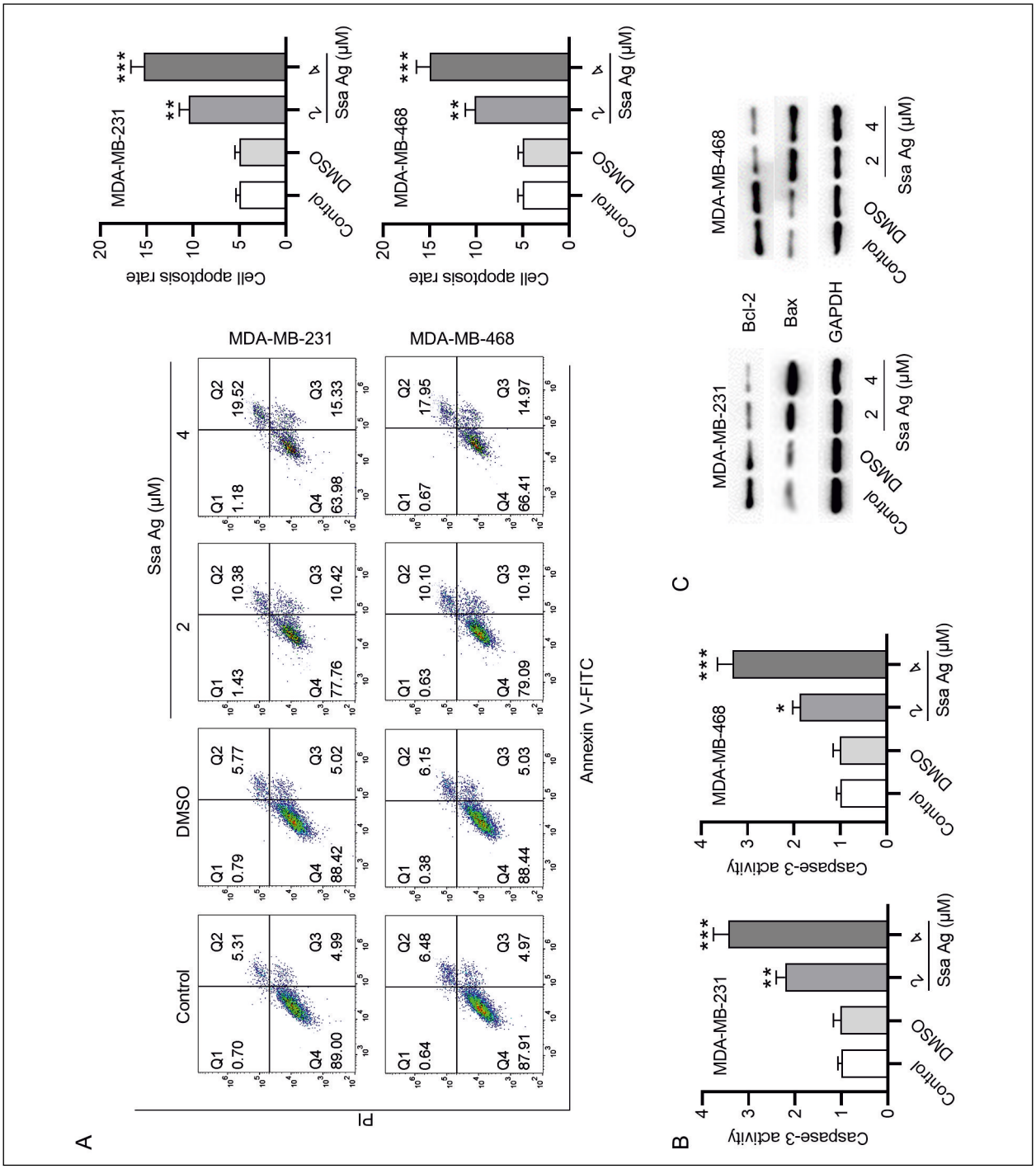

Figure 4. Ssa Ag promotes the apoptosis of TNBC cells. TNBC cells were treated with Ssa Ag (2 and 4 μM) for 24 h. **A.** TNBC cell apoptosis was assessed by flow cytometry analysis. **B.** Caspase-3 activity in TNBC cells was evaluated through caspase-3 activity analysis. **C.** The protein levels of apoptosis markers, Bcl-2 and Bax, in TNBC cells were analyzed through western blot. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

and inhibits the apoptosis of MDA-MB-468 cells through activating the p38 MAPK pathway [53]. The hyperactivation of the MEK/ERK pathway is related to the enhanced invasive and migratory capabilities of MDA-MB-231 and MDA-MB-468 cells [54]. Therefore, the activation of the MAPK pathway was demonstrated to promote the malignant behaviors of MDA-MB-468 and MDA-MB-231 cells. In addition, it has been reported that soyasaponin can attenuate diseases by modulating the MAPK signaling pathway [55]. Therefore, we speculated that Ssa Ag may modulate TNBC development and progression by modu-

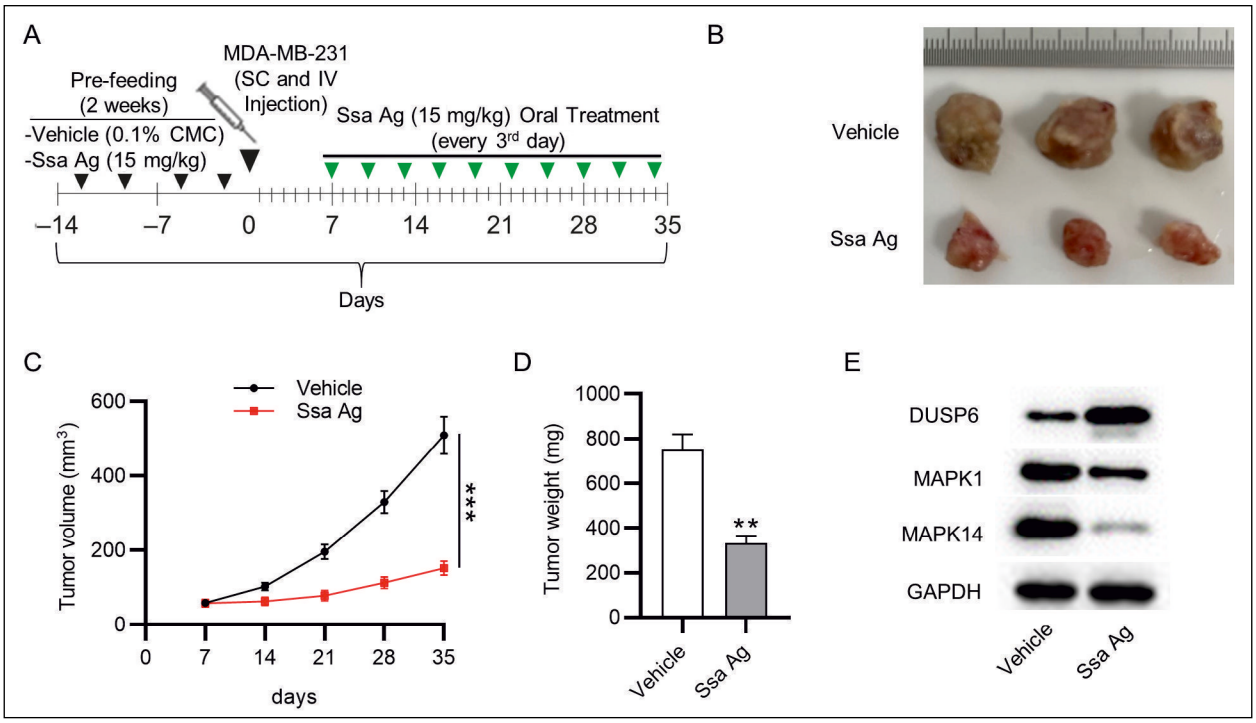

Figure 5. Ssa Ag suppresses tumor growth *in vivo*. **A.** The schematic model shows that the mice were pre-fed twice a week with vehicle (0.1% CMC) and Ssa Ag (15 mg/kg) for two weeks. Then, MDA-MB-231 cells were subcutaneously injected over the left and right thigh flanks. After one week, mice were fed regularly every other day for four weeks. **B.** The images of tumors dissected from mice were captured. **C.** The volume of tumors in vehicle and Ssa Ag groups was measured every 7 days. **D.** the weight of tumors in vehicle and Ssa Ag groups was measured after dissection. **E.** The protein levels of DUSP6, MAPK1, and MAPK14 in vehicle and Ssa Ag groups were evaluated through Western blot. $^{**}p < 0.01$, $^{***}p < 0.001$.

lating the DUSP6/MAPK pathway. Herein, with the help of GEPIA and Kaplan-Meier plotter, we know that DUSP6 is downregulated in BC tissues and brings poor prognosis of patients with BC. The high levels of MAPK1 and MAPK14 that participate in the MAPK signaling induce an unfavorable prognosis of patients with BC, indicating that DUSP6/MAPK pathway poses an endogenous influence on BC progression. Based on these data, we detected the expression of DUSP6, MAPK1, and MAPK14 in MDA-MB-468 and MDA-MB-231 cells. We discovered that DUSP6 was downregulated while MAPK1 and MAPK14 were upregulated in MDA-MB-468 and MDA-MB-231 cells versus normal cells. Moreover, the chemical structure of Ssa Ag that is pivotal to various biological functions was shown. Further, our study revealed that Ssa Ag upregulated the level of DUSP6 and downregulated the levels of MAPK1 and MAPK14 in TNBC cells, which suggests that Ssa Ag can restrain the malignant phenotypes of TNBC by inhibiting the DUSP6/MAPK signaling pathway.

Previously, the biological effects of soyasaponins in TNBC cells were widely investigated. For example, soyasaponin I was reported to attenuate the migratory capability of MDA-MB-231 cells, and soyasapogenin A was reported to suppress MDA-MB-231 cell proliferation and promote cell cycle arrest [17–19]. In this study, Ssa Ag was discovered to restrain the proliferation and facilitate the apoptosis of MDA-MB-468 and MDA-MB-231 cells, indicating the anti-tumor effects of Ssa Ag in TNBC, which is in line with the results in previous research. Importantly, an animal study was also performed to further verify that Ssa Ag inhibited tumor growth *in vivo via* upregulating the DUSP6 level and inactivating the MAPK pathway.

In summary, our study demonstrated that Ssa Ag suppresses TNBC cell proliferation and facilitates TNBC cell apoptosis as well as inhibits tumor growth by inactivating the DUSP6/MAPK pathway. Our findings provide new perspectives on the investigation of the biological effect of Ssa Ag on TNBC development and suggest that Ssa Ag might be practical for the treatment of patients with TNBC. In addition, more assays are needed for evaluating cell migratory and invasive capabilities to further validate the suppressive influence of Ssa Ag on TNBC development. Since our research focuses on the Ssa Ag role in TNBC, the role of Ssa Ag in other types of BC needs investigation in future studies.

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Conflicts of interest

The authors declare that there are no competing interests in this study.

References

1. Fahad Ullah M. Breast Cancer: Current Perspectives on the Disease Status. Adv Exp Med Biol. 2019; 1152: 51–64, doi: 10.1007/978-3-030-20301-6_4, indexed in Pubmed: 31456179.
2. Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and Mortality and Epidemiology of Breast Cancer in the World. Asian Pac J Cancer Prev. 2016; 17(S3): 43–46, doi: 10.7314/apjcp.2016.17.s3.43, indexed in Pubmed: 27165206.
3. Kolak A, Kamińska M, Sygit K, et al. Primary and secondary prevention of breast cancer. Ann Agric Environ Med. 2017; 24(4): 549–553, doi: 10.26444/aaem/75943, indexed in Pubmed: 29284222.
4. Carey LA, Dees EC, Sawyer L, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res. 2007; 13(8): 2329–2334, doi: 10.1158/1078-0432.CCR-06-1109, indexed in Pubmed: 17438091.
5. Sheng X, Dai H, Du Y, et al. LncRNA CARMN overexpression promotes prognosis and chemosensitivity of triple negative breast cancer via acting as miR143-3p host gene and inhibiting DNA replication. J Exp Clin Cancer Res. 2021; 40(1): 205, doi: 10.1186/s13046-021-02015-4, indexed in Pubmed: 34162418.
6. Boyle P. Triple-negative breast cancer: epidemiological considerations and recommendations. Ann Oncol. 2012; 23 Suppl 6: vi7–v12, doi: 10.1093/annonc/mds187, indexed in Pubmed: 23012306.
7. Stevens KN, Vachon CM, Couch FJ. Genetic susceptibility to triple-negative breast cancer. Cancer Res. 2013; 73(7): 2025–2030, doi: 10.1158/0008-5472.CAN-12-1699, indexed in Pubmed: 23536562.
8. De Cicco P, Catani MV, Gasperi V, et al. Nutrition and Breast Cancer: A Literature Review on Prevention, Treatment and Recurrence. Nutrients. 2019; 11(7), doi: 10.3390/nu11071514, indexed in Pubmed: 31277273.
9. Liyanage PY, Hettiarachchi SD, Zhou Y, et al. Nanoparticle-mediated targeted drug delivery for breast cancer treatment. Biochim Biophys Acta Rev Cancer. 2019; 1871(2): 419–433, doi: 10.1016/j.bbcan.2019.04.006, indexed in Pubmed: 31034927.
10. Yang SH, Tsatsakis AM, Tzanakakis G, et al. Soyasaponin Ag inhibits αMSHinduced melanogenesis in B16F10 melanoma cells via the downregulation of TRP2. Int J Mol Med. 2017; 40(3): 631–636, doi: 10.3892/ijmm.2017.3061, indexed in Pubmed: 28713957.
11. Kamo S, Suzuki S, Sato T. The content of soyasaponin and soyasapogenol in soy foods and their estimated intake in the Japanese. Food Sci Nutr. 2014; 2(3): 289–297, doi: 10.1002/fsn3.107, indexed in Pubmed: 24936299.
12. Tsai WT, Nakamura Y, Akasaka T, et al. Soyasaponin ameliorates obesity and reduces hepatic triacylglycerol accumulation by suppressing lipogenesis in high-fat diet-fed mice. J Food Sci. 2021; 86(5): 2103–2117, doi: 10.1111/1750-3841.15696, indexed in Pubmed: 33864648.
13. Shiraiwa M, Harada K, Okubo K. Composition and structure of „group B saponin" in soybean seed. Agric Biol Chem. 1991; 55(4): 911–917, indexed in Pubmed: 1368678.
14. Guang C, Chen J, Sang S, et al. Biological functionality of soyasaponins and soyasapogenols. J Agric Food Chem. 2014; 62(33): 8247–8255, doi: 10.1021/jf503047a, indexed in Pubmed: 25074337.
15. Xiao JX, Huang GQ, Zhang SH. Soyasaponins inhibit the proliferation of Hela cells by inducing apoptosis. Exp Toxicol Pathol. 2007; 59(1): 35–42, doi: 10.1016/j.etp.2007.02.004, indexed in Pubmed: 17582751.
16. Yang SH, Ahn EK, Lee JA, et al. Soyasaponins Aa and Ab exert an anti-obesity effect in 3T3-L1 adipocytes through downregulation of PPARγ. Phytother Res. 2015; 29(2): 281––287, doi: 10.1002/ptr.5252, indexed in Pubmed: 25366162.
17. Zhang W, Popovich DG. Chemical and biological characterization of oleanane triterpenoids from soy. Molecules. 2009; 14(8): 2959–2975, doi: 10.3390/molecules14082959, indexed in Pubmed: 19701138.
18. Hsu CC, Lin TW, Chang WW, et al. Soyasaponin-I-modified invasive behavior of cancer by changing cell surface sialic acids. Gynecol Oncol. 2005; 96(2): 415–422, doi: 10.1016/j.ygyno.2004.10.010, indexed in Pubmed: 15661230.
19. Omar A, Kalra RS, Putri J, et al. Soyasapogenol-A targets CARF and results in suppression of tumor growth and metastasis in p53 compromised cancer cells. Sci Rep. 2020; 10(1): 6323, doi: 10.1038/s41598-020-62953-5, indexed in Pubmed: 32286347.
20. Guo YJ, Pan WW, Liu SB, et al. ERK/MAPK signalling pathway and tumorigenesis. Exp Ther Med. 2020; 19(3): 1997–2007, doi: 10.3892/etm.2020.8454, indexed in Pubmed: 32104259.
21. Fang JY, Richardson BC. The MAPK signalling pathways and colorectal cancer. Lancet Oncol. 2005; 6(5): 322–327, doi: 10.1016/S1470-2045(05)70168-6, indexed in Pubmed: 15863380.
22. Sun QY, Ding LW, Johnson K, et al. SOX7 regulates MAPK/ERK-BIM mediated apoptosis in cancer cells. Oncogene. 2019; 38(34): 6196–6210, doi: 10.1038/s41388-019-0865-8, indexed in Pubmed: 31332289.
23. Chen L, Wang Y, Luan H, et al. DUSP6 protects murine podocytes from high glucoseinduced inflammation and apoptosis. Mol Med Rep. 2020; 22(3): 2273–2282, doi: 10.3892/mmr.2020.11317, indexed in Pubmed: 32705203.
24. Missinato MA, Saydmohammed M, Zuppo DA, et al. Dusp6 attenuates Ras/MAPK signaling to limit zebrafish heart regeneration. Development. 2018; 145(5), doi: 10.1242/dev.157206, indexed in Pubmed: 29444893.
25. Relav L, Estienne A, Price CA. Dual-specificity phosphatase 6 (DUSP6) mRNA and protein abundance is regulated by fibroblast growth factor 2 in sheep granulosa cells and inhibits c-Jun N-terminal kinase (MAPK8) phosphorylation. Mol Cell Endocrinol. 2021; 531: 111297, doi: 10.1016/j.mce.2021.111297, indexed in Pubmed: 33964319.

26. Wu F, McCuaig RD, Sutton CR, et al. Nuclear-Biased DUSP6 Expression is Associated with Cancer Spreading Including Brain Metastasis in Triple-Negative Breast Cancer. Int J Mol Sci. 2019; 20(12), doi: 10.3390/ijms20123080, indexed in Pubmed: 31238530.
27. Muda M, Boschert U, Dickinson R, et al. MKP-3, a novel cytosolic protein-tyrosine phosphatase that exemplifies a new class of mitogen-activated protein kinase phosphatase. J Biol Chem. 1996; 271(8): 4319–4326, doi: 10.1074/jbc.271.8.4319, indexed in Pubmed: 8626780.
28. Fan MJ, Liang SM, He PJ, et al. Dusp6 inhibits epithelial-mesenchymal transition in endometrial adenocarcinoma via ERK signaling pathway. Radiol Oncol. 2019; 53(3): 307–315, doi: 10.2478/raon-2019-0034, indexed in Pubmed: 31553703.
29. Hsu WC, Chen MY, Hsu SC, et al. DUSP6 mediates T cell receptor-engaged glycolysis and restrains T cell differentiation. Proc Natl Acad Sci U S A. 2018; 115(34): E8027––E8036, doi: 10.1073/pnas.1800076115, indexed in Pubmed: 30087184.
30. James NE, Beffa L, Oliver MT, et al. Inhibition of DUSP6 sensitizes ovarian cancer cells to chemotherapeutic agents via regulation of ERK signaling response genes. Oncotarget. 2019; 10(36): 3315–3327, indexed in Pubmed: 31164954.
31. Ma R, Ma L, Weng W, et al. DUSP6 SUMOylation protects cells from oxidative damage via direct regulation of Drp1 dephosphorylation. Sci Adv. 2020; 6(13): eaaz0361, doi: 10.1126/sciadv.aaz0361, indexed in Pubmed: 32232156.
32. Vo AH, Swaggart KA, Woo A, et al. Dusp6 is a genetic modifier of growth through enhanced ERK activity. Hum Mol Genet. 2019; 28(2): 279–289, doi: 10.1093/hmg/ddy349, indexed in Pubmed: 30289454.
33. Yang SH, Le B, Androutsopoulos VP, et al. Anti-inflammatory effects of soyasapogenol I-αa via downregulation of the MAPK signaling pathway in LPS-induced RAW 264.7 macrophages. Food Chem Toxicol. 2018; 113: 211–217, doi: 10.1016/j.fct.2018.01.050, indexed in Pubmed: 29407474.
34. Anbalagan M, Ali A, Jones RK, et al. Peptidomimetic Src/pretubulin inhibitor KX-01 alone and in combination with paclitaxel suppresses growth, metastasis in human ER/PR/HER2-negative tumor xenografts. Mol Cancer Ther. 2012; 11(9): 1936–1947, doi: 10.1158/1535-7163.MCT-12-0146, indexed in Pubmed: 22784709.
35. Luby AO, Subramanian C, Buchman LK, et al. Amifostine Prophylaxis in Irradiated Breast Reconstruction: A Study of Oncologic Safety In Vitro. Ann Plast Surg. 2020; 85(4): 424–429, doi: 10.1097/SAP.0000000000002110, indexed in Pubmed: 31850964.
36. Wiggins AKA, Kharotia S, Mason JK, et al. α-Linolenic Acid Reduces Growth of Both Triple Negative and Luminal Breast Cancer Cells in High and Low Estrogen Environments. Nutr Cancer. 2015; 67(6): 1001–1009, doi: 10.1080/01635581.2015.1053496, indexed in Pubmed: 26134471.
37. Yang SH, Tsatsakis AM, Tzanakakis G, et al. Soyasaponin Ag inhibits αMSHinduced melanogenesis in B16F10 melanoma cells via the downregulation of TRP2. Int J Mol Med. 2017; 40(3): 631–636, doi: 10.3892/ijmm.2017.3061, indexed in Pubmed: 28713957.
38. Omar A, Kalra RS, Putri J, et al. Soyasapogenol-A targets CARF and results in suppression of tumor growth and metastasis in p53 compromised cancer cells. Sci Rep. 2020; 10(1): 6323, doi: 10.1038/s41598-020-62953-5, indexed in Pubmed: 32286347.
39. Góral I, Wojciechowski K. Surface activity and foaming properties of saponin-rich plants extracts. Adv Colloid Interface Sci. 2020; 279: 102145, doi: 10.1016/j.cis.2020.102145, indexed in Pubmed: 32229329.
40. Güçlü-UstündağO, Mazza G. Saponins: properties, applications and processing. Crit Rev Food Sci Nutr. 2007; 47(3): 231–258, doi: 10.1080/10408390600698197, indexed in Pubmed: 17453922.
41. Ellington AA, Berhow M, Singletary KW. Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. Carcinogenesis. 2005; 26(1): 159–167, doi: 10.1093/carcin/bgh297, indexed in Pubmed: 15471899.
42. Ellington AA, Berhow MA, Singletary KW. Inhibition of Akt signaling and enhanced ERK1/2 activity are involved in induction of macroautophagy by triterpenoid B-group soyasaponins in colon cancer cells. Carcinogenesis. 2006; 27(2): 298–306, doi: 10.1093/carcin/bgi214, indexed in Pubmed: 16113053.
43. Hsu CC, Lin TW, Chang WW, et al. Soyasaponin-I-modified invasive behavior of cancer by changing cell surface sialic acids. Gynecol Oncol. 2005; 96(2): 415–422, doi: 10.1016/j.ygyno.2004.10.010, indexed in Pubmed: 15661230.
44. Lee IA, Park YJ, Joh EH, et al. Soyasaponin Ab ameliorates colitis by inhibiting the binding of lipopolysaccharide (LPS) to Toll-like receptor (TLR)4 on macrophages. J Agric Food Chem. 2011; 59(24): 13165–13172, doi: 10.1021/jf2033818, indexed in Pubmed: 22060784.
45. Pei Y, Zhao H, Du X, et al. [Apoptosis effects on human esophageal cancer cells by soyasaponin Bb and its machanism]. Wei Sheng Yan Jiu. 2010; 39(4): 444–446, indexed in Pubmed: 20726234.
46. Bermudez O, Pagğ G, Gimond C. The dual-specificity MAP kinase phosphatases: critical roles in development and cancer. Am J Physiol Cell Physiol. 2010; 299(2): C189–C202, doi: 10.1152/ajpcell.00347.2009, indexed in Pubmed: 20463170.
47. Hu X, Tang Z, Ma S, et al. Tripartite motif-containing protein 7 regulates hepatocellular carcinoma cell proliferation via the DUSP6/p38 pathway. Biochem Biophys Res Commun. 2019; 511(4): 889–895, doi: 10.1016/j.bbrc.2019.02.001, indexed in Pubmed: 30850165.
48. Keyse SM. Dual-specificity MAP kinase phosphatases (MKPs) and cancer. Cancer Metastasis Rev. 2008; 27(2): 253–261, doi: 10.1007/s10555-008-9123-1, indexed in Pubmed: 18330678.
49. Zhang Q, Fan XY, Guo WL, et al. The protective mechanisms of macroalgae Laminaria japonica consumption against lipid metabolism disorders in high-fat diet-induced hyperlipidemic rats. Food Funct. 2020; 11(4): 3256–3270, doi: 10.1039/d0fo00065e, indexed in Pubmed: 32219261.
50. Ramkissoon A, Chaney KE, Milewski D, et al. Targeted Inhibition of the Dual Specificity Phosphatases DUSP1 and DUSP6 Suppress MPNST Growth via JNK. Clin Cancer Res. 2019; 25(13): 4117–4127, doi: 10.1158/1078-0432.CCR-18-3224, indexed in Pubmed: 30936125.
51. Song H, Wu C, Wei C, et al. Silencing of DUSP6 gene by RNAi-mediation inhibits proliferation and growth in MDA-MB-231 breast cancer cells: an in vitro study. Int J Clin Exp Med 2015. 8(7): p. : 10481–90.
52. Wu X, Chen S, Lu C. Amyloid precursor protein promotes the migration and invasion of breast cancer cells by regulating the MAPK signaling pathway. Int J Mol Med. 2020; 45(1): 162–174, doi: 10.3892/ijmm.2019.4404, indexed in Pubmed: 31746365.

53. Park S, Han SH, Kim HG, et al. PRPF4 is a novel therapeutic target for the treatment of breast cancer by influencing growth, migration, invasion, and apoptosis of breast cancer cells via p38 MAPK signaling pathway. Mol Cell Probes. 2019; 47: 101440, doi: 10.1016/j.mcp.2019.101440, indexed in Pubmed: 31445970.
54. Nokin MJ, Bellier J, Durieux F, et al. Methylglyoxal, a glycolysis metabolite, triggers metastasis through MEK/ERK/SMAD1 pathway activation in breast cancer. Breast Cancer Res. 2019; 21(1): 11, doi: 10.1186/s13058-018-1095-7, indexed in Pubmed: 30674353.
55. Yang SH, Le B, Androutsopoulos VP, et al. Anti-inflammatory effects of soyasapogenol I-αa via downregulation of the MAPK signaling pathway in LPS-induced RAW 264.7 macrophages. Food Chem Toxicol. 2018; 113: 211–217, doi: 10.1016/j.fct.2018.01.050, indexed in Pubmed: 29407474.

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