Magnolin Inhibits Proliferation and Invasion of Breast Cancer MDA-MB-231 Cells by Targeting the ERK1/2 Signaling Pathway

Jing Wang,⁎,⁎⁎ Shengchu Zhang⁎,⁎⁎⁎ Kuo Huang,⁎Lang Shi,⁎ and Qingyong Zhang⁎

⁎Department of Thyroid and Breast Surgery, The First College of Clinical Medical Science, China Three Gorges University; Yi Ling Road 183, Yichang, Hubei 443003, P. R. China; ⁎Department of Clinical Laboratory, The First College of Clinical Medical Science, China Three Gorges University; Yi Ling Road 183, Yichang, Hubei 443003, P. R. China; and ⁎⁎The First College of Clinical Medical Science, China Three Gorges University; Yi Ling Road 183, Yichang, Hubei 443003, P. R. China.

Received September 24, 2019; accepted January 8, 2020

The aim of this study was to evaluate the effects of Magnolin (MGL) on inhibition of human breast cancer cells, and explore the underlying molecular mechanisms. The viability of the treated cells was assessed with the Cell Counting Kit-8 (CCK-8) assay, and the proliferation was analyzed in terms of EdU uptake, colony formation, and flow cytometry. The in vitro invasion and migration were determined by the transwell and wound healing assays respectively. The mRNA and protein levels of relevant factors was evaluated by quantitative real-time PCR and Western blotting respectively. MGL significantly decreased the viability and promoted apoptosis of MDA-MB-231 cells, along with reducing EdU incorporation rate as well as the colony forming capacity compared to the untreated control cells. In addition, the in vitro invasion and migration were also significantly inhibited by MGL. Furthermore, MGL suppressed the phosphorylation of MEK1/2, extracellular signal-regulated kinase (ERK)1/2 and significantly downregulated the expression of cyclin-dependent kinase 1 (CDK1), the anti-apoptotic B-cell lymphoma 2 (BCL2) and metastasis-associated matrix metalloproteases (MMPs) 2 & 9, and upregulated the cleaved caspases 3 and 9. After ERK was completely inhibited with the small interfering RNA (siRNA), MGL had no effect on these factors, indicating that ERK is essential for MGL action in breast cancer. In conclusion, MGL inhibits proliferation and invasion of and induces apoptosis in breast cancer cells through the ERK pathway.

Key words Magnolin; breast cancer; proliferation; extracellular signal-regulated kinase (ERK)1/2; apoptosis; invasion

Introduction

Breast cancer is the most commonly diagnosed cancer, and the leading cause of cancer-related mortality in women.1) Mammography and screening for the tumor markers carcino-embryonic antigen (CEA) and cancer antigen (CA) are routinely used to identify women at high risk. However, due to their low sensitivity and specificity, these methods have limited diagnostic utility.2,3) In addition, due to the high risk of recurrence and metastasis, the prognosis of breast cancer is generally poor.4,5) Therefore, there is an urgent need to develop new diagnostic strategies and therapies to improve the prognosis of breast cancer patients.

In recent years, natural products have gained considerable attention as anti-cancer drugs due to their high efficacy and low toxicity.6,7) Magnolin (MGL), an active lignan isolated from the bark of Magnolia biondii, has anti-inflammatory activity, anti-cancer, antioxidant and vasodilation effects.8,9) It has traditionally been used for treating headaches, nasal congestion, inflammation and vasodilation (Chang et al.; Ibarra-Alvarado et al.; Kim et al.; Hou et al.). Recent studies show that MGL significantly inhibits cancer cell proliferation and transformation by targeting extracellular signal-regulated kinase (ERK),10) a key signaling molecule involved in malignant transformation11,12) and cancer cell metastasis.13,14) Mechanistically, it inhibits the production of tumor necrosis factor-α (TNF-α) and prostaglandin E2 (PGE2),15) and the pro-tumor epidermal growth factor (EGF)6) by blocking the ERK1/2 pathway. Although its potent bioactivity and low toxicity make MGL a promising drug candidate against breast cancer, the molecular mechanisms underlying its anti-proliferative and anti-invasive effects remain to be elucidated.

The aim of this study was to investigate the potential anti-neoplastic effects of MGL in an in vitro model of human breast cancer, and dissect the mechanistic basis. We found that MGL down-regulated several anti-apoptotic, proliferative and metastatic proteins through the ERK pathway, and up-regulated the pro-apoptotic proteins, to inhibit the migration and invasion of breast cancer cells.

Experimental

Cell and Drug Sources MDA-MB-231, which is a triple negative breast cancer cell line, often appears as an experimental cell line in the case of triple negative breast cancer.17,18) Triple-negative breast cancer is a basal-like breast cancer caused by lack of estrogen receptor, progesterone receptor and HER2 expression.19) The MDA-MB-231 cell line was purchased from the American Type Culture Collection (ATCC), and maintained in the Dulbecco’s modified Eagle’s medium-high glucose (H-DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco, NY, U.S.A.), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco BRL, U.S.A.) at 37°C under 5% CO2. The medium was changed every 2–3 d. Magnolin
Viability Assay and Treatment Protocol  MDA-MB-231 cells were seeded into 96-well plates at the density of $3 \times 10^4$ cells/cm² and cultured for 24 h in complete medium. The cells were then starved for 24 h in serum-free medium, followed by 48 h treatment with different concentrations of MGL (10, 20, 30, 50, and 100 $\mu$M), or with 50 $\mu$M MGL for 1, 2, 3, 4, 5, 6, and 7 d in complete medium. The viability was evaluated using Cell Counting Kit-8 (CCK-8 assay), and OD at 450 nm was measured. Based on the results, cells were treated with 20 and 50 $\mu$M MGL for 24 or 48 h as described above for the subsequent experiments. In addition, the cells were also treated with 10 $\mu$M SCH772984 (ERK1/2 inhibitor) or the small interfering RNA (siRNA)-ERK along with MGL stained with 100 $\mu$M EdU labeling mixture to grow till confluent. A linear wound measuring 10 mm was made by scratching the monolayer with a sterile pipette tip, and the cellular debris were removed by washing with PBS. Fresh complete medium with or without MGL (20 and 50 $\mu$M) was added, and images were taken at 0, 24, and 48 h post-wounding. The number of cells that migrated to the wound site were counted.

Wound Healing Assay  The cells were seeded at a density of 200 cells per plate in a volume of 2 mL complete medium. Then, they are maintained in the culture for 6 and 12 d. The medium was then discarded and the cells were fixed with methanol for 30 min, and stained with diluted crystal violet. The plates were observed under a microscope, and colonies consisting of >50 cells were counted.

Quantitative RT-PCR  Total RNA was extracted using RNA Simple Total RNA Kit according to the manufacturer’s instructions, and quantified in terms of absorbance at 260 nm with a spectrophotometer. RevertAid™ First Strand cDNA synthesis Kit (Thermo Fisher Scientific, Waltham, MA, U.S.A.) was used to synthetize the first-strand cDNA. The expression levels of cyclin-dependent kinase 1 (CDK1), B-cell lymphoma 2 (BCL2), caspase 3/9, matrix metalloproteinase 2/9 (MMP2/9) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs were analyzed by qRT-PCR in Bio-rad Transwell Assay  The invasion of MGL-treated MDA-MB-231 cells were evaluated using the transwell assay. Matrigel-coated transwell chambers were placed in 24-well plates, and the upper chambers were seeded with 5 $\times 10^4$ cells per well in 500 $\mu$L serum-free medium with or without MGL (20 and 50 $\mu$M). The lower chambers were filled with 600 $\mu$L complete medium, and the cells were cultured for 12, 24, or 48 h. The cells remaining on the upper surface of the membrane were removed with a cotton swab, and the cells that had migrated to the lower surface were fixed, stained with 4',6-diamidino-2-phenylindole (DAPI) and counted. In order to avoid interference of apoptotic cells on cell migration and invasion, we performed an additional transwell assay. Briefly, after treatment with 0, 20, and 50 $\mu$M MGL for 24 h, apoptotic MDA-MB-231 cells were removed. Then the equal density of the cells was performed the above transwell assay protocol (Supplementary Fig. 1a).

Colony Formation Assay  The cells were seeded into 6-well plates at the density of $5 \times 10^4$ cells per well, and allowed to grow till confluent. A linear wound measuring 10 mm × 1.4 mm was made by scratching the monolayer with a sterile pipette tip, and the cellular debris were removed by washing with PBS. Fresh complete medium with or without MGL (20 and 50 $\mu$M) was added, and images were taken at 0, 24, and 48 h post-wounding. The number of cells that migrated to the wound site were counted.

Colonies of 50 cells were counted.

Flow Cytometry Assay  After harvested, the cells were washed using cold PBS. Then they are resuspended at the density of 1 $\times 10^6$ cells/mL in PBS. The cell suspension was centrifuged, and the pellet was fixed with 100 $\mu$L 30% methanol for 30 min. Propidium iodide (PI) was then added per tube for detecting cell cycle. Apoptosis were performed by Annexin V/PI staining. The stained cells were detected using BD FACS Calibur Flow Cytometer (BD Accuri C6; Becton Dickinson Corp.) at 488 nm excitation wavelength.

Table 1. List of qPCR Primers

| Gene symbol | Accession number | Primer sequence (3′→5′) | Size (bp) |
|-------------|------------------|--------------------------|----------|
| CDK1        | NM_001786.4      | CTTGGCTTTCAAAGCTGGCTC   | 570      |
|             |                  | GCTCTTGCAAGGGCAAAAAATC  |          |
|             |                  | CTTTGGTTGCGTGGGGTGCA    | 187      |
| BCL2        | NM_000633.2      | GAAATCAAACAGAGGCCGCA     |          |
|             |                  | ATTAGCGCCTCCATCGTAGC     |          |
| CASP3       | NM_032991.2      | TCACCATTGCGTCAAGACAC    | 402      |
| CASP9       | NM_001278054.1   | TGGGACACTCGAGGGAGTCAG    | 247      |
| MMP2        | NM_004530.6      | TGGTCTTCTGTCGCTGACATCA  | 293      |
| MMP9        | NM_004994.3      | CATCCGCGACCTCTATGTC     | 230      |
| GAPDH       | NM_002046.7      | GCTCTCTGCTTCTCGTTC      | 273      |
CFX96TM Real-Time PCR system (Bio-Rad, Hercules, CA, U.S.A.) using PCR Master Mix Kit (Promega, Madison, WI, U.S.A.). GAPDH was used as the internal control. The primer sequences are shown in Table 1.

**Western Blotting**  The treated cells were lysed with RIPA buffer supplemented with protease inhibitors. Equal amounts (40 µg) of protein per sample were resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The latter were blocked with Tris buffered saline (TBS) containing 5% non-fat milk powder at 37°C for 30 min, and then incubated overnight with primary antibodies against CDK1 (Abcam: ab32094, 1:1000), BCL-2 (Abcam: ab185002, 1:1000), cleaved caspase 3 (Abcam: ab32042, 1:1000), cleaved caspase 9 (Abcam: ab23234, 1:1000), MMP2 (Abcam: ab92536, 1:1000), MMP9 (Abcam: ab76003, 1:1000), p-ERK1/2 (Abcam: ab201015, 1:1000), ERK1/2 (Abcam: ab36991, 1:1000), MEK1/2 (Abcam: ab178876, 1:1000), p-MEK1/2 (Abcam: ab194754, 1:1000), β-actin (Abcam: ab119716, 1:1000), and GAPDH (Abcam: ab181602, 1:1000) at 4°C. Following by 1 h incubation with the corresponding secondary antibody at 37°C, the immuno-reactive bands were developed using Supersignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific), and analyzed using Quantity One Software (Bio-Rad).

**Ethics and Consent**  All studies meet medical ethical requirements in China Three Gorges University.

**Statistical Analyses**  All experiments were performed at least four times. Descriptive data were presented as the mean ± standard deviation (S.D.), and compared by one-way ANOVA using Origin 8.0 software (OriginLab, Northampton, MA, U.S.A.). *p* < 0.05 was considered statistically significant.

**Results**

**MGL Inhibits Proliferation of MDA-MB-231 Cells**

MGL significantly reduced the viability of MDA-MB-231 cells in a dose-dependent manner, with IC50 of 30.34 µM (Fig. 1a). Subsequent experiments were therefore conducted with 50 µM for 24 h and longer (Fig. 1b). Furthermore, MGL also reduced EdU incorporation in a dose-dependent manner (Fig. 1c), and decreased the number of proliferating cells from 37.78 ± 3.23 to 17.89 ± 4.28 at the dose of 50 µM (Fig. 1d). Consistent with

Fig. 1.  MGL Inhibited the Cell Proliferation of MDA-MB-231

(a) Cell viability was significantly inhibited by MGL in a dose-dependent manner at concentration ranging from 10 to 100 µM, the IC50 was calculated. (b) Cell proliferation curve of control (DMSO) and MGL (50 µM) treatment group for 7 d. (c) DNA synthesis for MGL (50 µM) treatment for 48 h. (d) Statistical comparison of EdU positive count of MGL treatment (20 and 50 µM) versus to control. (e and f) Flow cytometry assay for cell cycle with MGL (50 µM) treatment for 24 and 48 h. * and # *p* < 0.05 were accepted as significant difference.
this, treatment with 50 µM MGL for 24 or 48 h significantly decreased the proportion of MDA-MB-231 cells in the S-phase and G2/M phase whereas increased the proportion of G1 phase compared to that in the control cells (Figs. 1e, f).

**MGL Inhibits the in Vitro Invasion and Migration of MDA-MB-231 Cells** The primary reason for breast cancer recurrence is the high rate of cell migration and the ensuing tumor metastasis. As shown in Figs. 2a and b, 20 and 50 µM MGL decreased the number of invading cells across the transwell membrane from 83.74 ± 6.89 to 43.17 ± 3.24 and 11.42 ± 4.96, respectively (Fig. 2b). The wound healing assay also showed that the number of migrating MDA-MB-231 cells into the wound site was significantly reduced by MGL treatment (Figs. 2c and d). In order to avoid interference of apoptotic cells on cell migration and invasion, we performed an additional transwell assay. The final data showed that 20 and 50 µM MGL still significantly inhibited cell invasion of MDA-MB-231 cells (Supplementary Fig. 1b), but the differences were smaller compared with our previous results. The relevant result was shown as below (Supplementary Fig. 1c). After treated with 20 and 50 µM MGL, the number of invading cells was decreased from 40.00 ± 2.58 to 33.50 ± 3.11 (*p < 0.05, 20 µM vs. control) and 21.75 ± 2.50 (*p < 0.05, 50 µM vs. control), respectively.

**MGL Inhibited the Colony Formation of the MDA-MB-231 Cells** The colony forming ability of the MGL-treated cells was also evaluated as another index of proliferative capacity. As shown in Figs. 2e and f, compared to the untreated control, 50 µM MGL significantly decreased the colony numbers from 237.14 ± 51.26 to 92.43 ± 38.26 on day 6, and from 578.61 ± 28.87 to 321.23 ± 48.56 on day 12. Taken together, MGL has a potent inhibitory effect on human breast cancer cells.

**MEK-ERK1/2 Is the Targeting Pathway Involving MGF Regulating Cell Behavior of the MDA-MB-231 Cells** To determine the mechanistic basis of MGL action, we analyzed the expression levels of various critical factors affecting proliferation, metastasis and apoptosis of cancer cells.
MGL significantly decreased the levels of CDK1 and the pro-metastatic MMPs 2 and 9 (Figs. 3a–c). The anti-apoptotic protein BCL-2 was also significantly downregulated, and the pro-apoptotic cleaved caspases 3 and 9 were up-regulated by MGL treatment (Figs. 3b, c). In addition, and consistent with previous findings, MGL also significantly reduced MERK1/2, p-MEK1/2, ERK1/2 and p-ERK1/2 expression levels (Fig. 4), indicating that it likely modulates the above factors via the MEK-ERK1/2 pathway. To confirm this hypothesis, we blocked ERK1/2 pharmacologically (with SCH772984) or genetically (siRNA-ERK) in the breast cancer cells along with MGL treatment. SCH772984 further augmented the inhibitory effect of MGL on BCL-2, CDK1, and p/t-ERK compared to MGL treatment alone (Fig. 4). The total knockdown of ERK on the other hand made MGL redundant, with no additional effects on the aforementioned factors (Fig. 4). These results clearly indicate the involvement of the ERK signaling pathway in the abovementioned effects of MGL on MDA-MB-231 cells.

**Discussion**

Although MGL is effective against various cancers, its molecular targets have not yet been identified. In a recent study, MGL inhibited the growth of prostate cancer cells in an in vitro cell culture system and an animal transplantation model. Some studies have reported ERK1 and ERK2 as the molecular targets of MGL, although the specific molecular mechanism is still unclear. ERK1/2 is a member of the mitogen-activated protein kinase (MAPK) superfamily and mediates cell proliferation and apoptosis. The Ras-Raf-MEK-ERK signaling cascade is a well-studied proliferative pathway, but the mechanisms underlying ERK1/2-mediated cell death are largely unknown.

Our results indicate that MGL reduces breast cancer cell proliferation and metastasis by inhibiting ERK phosphorylation, thereby blocking the anti-apoptotic BCL-2, CDK1, MMP2 and MMP9, and activating the caspases 3 and 9. ERK knockdown, however, desensitized the cells to these molecular effects of MGL, indicating that MGL requires ERK to exert
its effects in breast cancer cells. Our findings are consistent with a previous study that showed that MGL induced apoptosis via ERK inactivation and BCL-2 de-phosphorylation. BCL-2 interacts with MAPK-ERK, which is the classic driver of the G1-S phase of the cell cycle, and ERK1/2 phosphorylation up-regulates the BCL-2 family of anti-apoptotic proteins, including BCL2, Bcl-X1 and Mcl-1, and inactivates the pro-apoptotic proteins depending on the cell type. Studies have also shown that MGL can directly up-regulate Bax and down-regulate BCL-2. MGL may inhibit MMP2 and MMP9 activity by ERK phosphorylation. Additional stimulation can up-regulate MMP2 and MMP9 expression by ERK phosphorylation, leading to ECM degradation and cancer cell invasion.

MGL regulates caspase protein activity via the MEK-ERK-BCL2 axis. ERK is the downstream of MEK and has emerged as an important tumor influencing factor in the studies of cancer. In breast cancer, inhibition of phosphorylation of ERK can inhibit breast cancer migration. It has been shown that inhibition of ERK pathway expression can suppress breast cancer migration/invasion. ERK can control caspase-9 activity through BCL2. Inhibition of CDK1 activity may be through the ERK pathway. In addition to the cell cycle, CDK1 also regulates apoptosis by affecting caspase protein activity. A variety of stimuli, including oncogenes, cellular stress, DNA damaging agents, and apoptotic processes under the action of many chemotherapeutic drugs are involved in the initiation of caspase-9. Caspase-9 is activated in apoptotic bodies formed by the release of Apaf-1 from mitochondria in

Fig. 4. MGL Inhibited MDA-MB-231 Tumorigenesis via MEK-ERK1/2 Pathways
(a) Western blotting for phosphorylated MEK1/2, total MEK1/2 of the cells with 10, 50, 100µM MGL. (b) Semi-quantification analysis, (*) p < 0.05 was accepted as statistically significant. (c) Immunofluorescent staining of phosphorylated MEK1/2 with different treatments. (d) Western blotting for BCL2, CDK1, phosphorylated ERK1/2, total ERK1/2 of the cells with 50 and 100µM MGL, 1µM SCH772984 (ERK inhibitor), and ERK si-RNA (siR-ERK) treatment for 48h. (e–g) Statistical analysis of the target proteins were collected by the gray semi-quantification and presented ratio to β-actin. (*) p < 0.05 and (#) p < 0.05 were accepted as statistically significant.
response to cytochrome c.\textsuperscript{22} The release of cytochrome c in mitochondria is controlled by BCL2 family proteins. Once activated, caspase-9 cleaves and activates a related caspase such as caspase-3,\textsuperscript{23} which targets a variety of cellular components to disrupt cells and present fragments for phagocytosis.

Conclusion

MGL inhibits breast cancer cell growth by blocking the ERK1/2 pathway, and consequently downregulating pro-proliferative, anti-apoptotic and pro-metastasis factors, and upregulating the pro-apoptotic factors. Further animal and clinical studies need to be conducted before incorporating MGL in anti-breast cancer therapy.

Acknowledgments

This work was supported by China Three Gorges University. No sample of human tissue is involved in the study.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Materials

The online version of this article contains supplementary materials.

References

1) Waks A. G., Winer E. P., \textit{JAMA}, 321, 288–300 (2019).
2) Guan X., Ma F., Li C., Wu S., Hu S., Huang J., Sun X., Wang J., \textit{Cancer Commun.} (Lond.), 39, 1 (2019).
3) Aliwaini S., Lubbad A. M., Shourfa A., Hamada H. A. A., Ayesh A., \textit{Cancer Med. Cell. Longev.}, 5 (2019).
4) Wang K., Li H. L., Xiong Y. F., Shi Y., Li Z. Y., Li J., Zhang X., Li H. Y., \textit{Cancer Medicine}, 8, 686–700 (2019).
5) Almirzae S., Bagherzadeh M., Akbari M. R., \textit{Clin. Genet.}, 95, 643–660 (2019).
6) Fatima I., Kanwal S., Mahmood T., \textit{Dose-Response}, 17, 155932818183227 (2019).
7) Faustino C., Francisco A. P., Isca V. M. S., Duarte N., \textit{Curr. Pharm. Des.}, 24, 4270–4311 (2018).
8) Kim J. Y., Lim H. J., Lee Y., Kim J. S., Kim D. H., Lee H. J., Kim H. D., Jeon R., Ryu J. H., \textit{Bioorg. Med. Chem. Lett.}, 19, 937–940 (2009).
9) Wang F., Zhang G., Zhou Y., Gui D., Li J., Xing T., Wang N., \textit{Oxid. Med. Cell. Longev.}, 2014, 2034584 (2014).
10) Lee C. J., Lee M. H., Yoo S. M., Choi K. I., Song J. H., Jang J. H., Oh S. R., Ryu H. W., Lee H. S., Surh Y. J., Cho Y. Y., \textit{BMC Cancer}, 15, 576 (2015).
11) Mu P., Liu K., Lin Q., Yang W., Liu D., Lin Z., Shao W., Ji T., \textit{Onco-ology Letters}, 17, 1445–1452 (2019).
12) Zhou G., Yang J., Song P., \textit{Oncology Letters}, 17, 2266–2270 (2019).
13) Zhang W., Hua T., Li J., Zheng L., Wang Y., Xu M., Qi G., \textit{American Journal of Translational Research}, 10, 3723–3732 (2018).
14) Cheng D., Li J., Zhang L., Hu L., \textit{Oncology Letters}, 17, 1363–1371 (2019).
15) Baek J. A., Lee Y. D., Lee C. B., Go H. K., Kim J. P., Seo J. J., Rhee Y. K., Kim A. M., Na D. J., \textit{Biol. Chem.}, 20, 122–128 (2009).
16) Zhang J., Guo L., Zhang Q., Liu K., Dong Z., \textit{Med.-Rep.}, 18, 5215–5220 (2018).
17) Illiano M., Sapio L., Salzillo A., Capasso L., Caiata I., Chiosi E., Spina A., Naviglio S., \textit{Biochem. Pharmacol.}, 152, 104–113 (2018).
18) Varghese E., Samuel S. M., Varghese S., Cheema I., Mamtani R., Busselberg D., \textit{Biomolecules}, 8, 163 (2018).
19) Guney Eskilier G., Cecener G., Egeki U., Tunca B., \textit{Acta Pathologica, Microbiologica, et Immunologica Scandinavica}, 126, 371–379 (2018).
20) Huang Y., Zou X., Zhang X., Wang F., Zhu W., Zhang G., Xiao J., Chen M., \textit{Biomed. Pharmacother.}, 87, 714–720 (2017).
21) Lee C. J., Lee H. S., Ryu H. W., Lee M. H., Lee J. Y., Li Y., Dong Z., Lee H. K., Oh S. R., Cho Y. Y., \textit{Carcinogenesis}, 35, 432–441 (2014).
22) Song H., Lee C. J., An H. J., Yoo S. M., Kang H. C., Lee J. Y., Kim K. D., Kim D. J., Lee H. S., Cho Y. Y., \textit{Mol. Carcinog.}, 58, 88–101 (2019).
23) Wang Y., Wang C., Liu N., Hou J., Xiao W., Wang H., \textit{FASEB J.}, 33, 3901–3911 (2019).
24) Tessoulin B., Papin A., Gomez-Bougie P., Bellanger C., Amiot M., Pellat-Deceunynck C., Chiron D., \textit{Frontiers in Oncology}, 8, 645 (2018).
25) Cho J. H., Hong W. G., Jung Y. J., Lee J., Lee E., Hwang S. G., Um H. D., Park J. K., \textit{The Journal of the International Society for Oncodevelopmental Biology and Medicine}, 37, 7315–7325 (2016).
26) Sun Q., Liang Y., Zhang T., Wang K., Yang X., \textit{Biochem. Biophys. Res. Commun.}, 487, 625–632 (2017).
27) Liu X., Zhao W., Wang W., Lin S., Yang L., \textit{Biomed. Pharmacother.}, 92, 429–436 (2017).
28) Lee W. T., Lee T. H., Cheng C. H., Chen K. C., Chen Y. C., Lin C. W., \textit{An International Journal Published for the British Industrial Biological Research Association}, 78, 33–41 (2015).
29) Zhang P., Kawakami H., Liu W., Zeng X., Streibhardt K., Tao K., Huang S., Sinicrope F. A., \textit{MCR}, 16, 378–389 (2018).
30) Chan Y. W., Ma H. T., Wong W., Ho C. C., On K. F., Poon R. Y., \textit{Oncol. Rep.}, 34, 2776–2781 (2015).
31) Yan J., Ma C., Cheng J., Li Z., Liu C., \textit{Oncol. Letters}, 17, 2266–2270 (2019).
32) Kim J. Y., Kim Y. J., Seok K. O., Lee S., Park J. H., \textit{Mol. Cell. Biol.}, 77, 320–327 (2010).
33) Fujita E., Egashira J., Urase K., Kuida K., Momoi T., \textit{Cell Death Differ.}, 8, 335–344 (2001).