Current Topics in Peptide & Protein Research
Vol. 23, 2022

Original Article

Autocrine motility factor and octyl gallate synergistically control melanoma cell proliferation *via* induced suppression of glucose-6-phosphate dehydrogenase

Se Gie Kim[1] and Hee Sung Park[2,*]

[1]Department of Pharmaceutical Engineering, Daegu Catholic University, Gyeongsan, Korea;
[2]Department of Biomedical Science, Daegu Catholic University, Gyeongsan, Korea.

ABSTRACT

Autocrine motility factor (AMF) acts as a growth inhibitor as well as activator of proliferating cancer cells. This study demonstrated that AMF significantly inhibited melanoma cells by downregulation of the mRNA and protein expression of glucose-6-phosphate dehydrogenase (G6PD) and hypoxia-inducible factor (HIF-1α). Moreover, the synergy between activities of AMF and cytotoxic octyl gallate (OG) was observed.

KEYWORDS: autocrine motility factor, glucose-6-phosphate dehydrogenase, hypoxia-inducible factor, melanoma cells, octyl gallate.

INTRODUCTION

BRAF/MEK inhibitors and anti-PD-1/CTLA-4 monoclonal antibodies have been well appreciated as the first-line classes of melanoma agents. However, resistance to these immune and targeted melanoma therapies remains a severe challenge [1-3]. To improve this, considerable attention has been given to the use of human-derived oncotoxic proteins, such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and human α-lactalbumin made lethal to tumor cells (HAMLET) [4, 5]. Additionally, secreted AMF could be a noteworthy alternative agent [6, 7].

*Corresponding author: hspark@cu.ac.kr

Glucose-6-phosphate isomerase (GPI) interconverts glucose-6-phosphate and fructose-6-phosphate during glycolysis. AMF, an extracellular form of GPI, promotes cancer cell motility and proliferation by activating the AKT and/or ERK pathways [8]. AMF also plays an anti-apoptotic role, ameliorating ER stress [9, 10]. Overexpression of AMF is frequently observed in many cancers [11, 12]. Intriguingly, AMF is able to suppress cancer cell growth in an AMF-type and dose-dependent manner but further insights into the mechanism of action of AMF are required [6]. Meanwhile, AMF has been proposed as a biological sensitizer to augment the cytotoxicity of phytochemicals to reduce drug resistance and elevate therapeutic benefits [6, 7].

Octyl gallate (OG) has been used as a preservative in foods and cosmetics. Further, OG has attracted attention due to its multiple biological functions, such as antiviral, antifungal, antibacterial, antioxidant, anti-inflammatory, and anti-cancer activities [13-15]. OG is cell permeable and highly bioavailable [16].

We have tested the effectiveness of AMF alone or in combination with OG to control growth of melanoma cells. Herein we demonstrated that AMF from A549 lung cancer cells, identical to the human GPI/AMF, significantly impaired melanoma cell proliferation by downregulating the expression of G6PD and HIF-1α mRNA and protein. Furthermore, we found that AMF and OG exerted a synergistic effect against melanoma cell proliferation.

MATERIALS AND METHODS

Cell culture and cell viability assay

Human SKMEL-2 and murine B16-F10 melanoma cells (KCLB, Korea) were maintained in DMEM containing 10% heat-inactivated fetal bovine serum and 1% antibiotics (penicillin/streptomycin). Cell viability was analyzed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assays.

Quantitative real-time PCR

RNA was extracted using RNeasy Mini Kit (Qiagen, Germany) and cDNA was synthesized using SuperScript III cDNA Synthesis Kit (Invitrogen, USA). Quantitative PCR was performed with Bio-Rad SYBR Green Supermix (Bio-Rad, USA) and specific mRNA amounts were calculated in relation to β-actin mRNA. Primers used are as follows: HIF-1α, forward ACAGTATTCCAGCAGACTCAA and reverse CCTACTGCTTGAAAAAGTGAA; hexokinase II (HK2), forward GGATGATTGCC TCGCATCTGC and reverse GGAACTCTCCGT GTTCTGTCC; aldolase A (ALDA), forward CCATGCCCTACCAATATCCAGC and reverse GGTGGTAGTCTCGCCATTTGTCC; GPI, forward AGGCTGCTGCCACATAAGGT and reverse AGCGTCGTGAGAGGTCACTTG; G6PD, forward AAACGGTCGTACACTTCGGG and reverse GGTAGTGGTCGATGCGGTAG; β-actin, forward CATGTACGTTGCTATCCAGGC and reverse CTCCTTAATGTCACGCACGAT.

Recombinant protein production

RNA was extracted using RNeasy Mini Kit (Qiagen, Germany) and cDNA was synthesized using SuperScript III cDNA Synthesis Kit (Invitrogen, USA). AMF cDNA was cloned by PCR using using the forward primer (5'-TACATATGGCCGCTC TCACCCGGGACCCCCAGTTCCAGAA-3') and the reverse primer (5'-ATCTCGAGTTATTGG ACTCTGGCCTCGCGCTGCT-3'). PCR products were cloned into the pCold I DNA (Takara, Korea). *Escherichia coli* BL21 cells harboring AMF cDNA were grown overnight in LB medium containing ampicillin (100 μg/mL) and further subjected to treatment with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) according to the manual of the pCold I DNA cold-shock expression system. Harvested cells in lysis buffer (20 mM Tris–HCl pH 8.0, 200 mM NaCl, 1 mM DTT, and 0.5 mM PMSF) were disrupted using a French pressure cell press (Thermo IEC, USA). From total soluble fractions, recombinant AMF proteins were purified using His60 Ni resin affinity chromatography (Promega, USA). Proteins were quantified using the Bio-Rad protein assay reagent.

Cell stress tolerance assay

Melanoma cells at 100% confluency were treated with AMF and/or OG for 36 h, washed with PBS solution, and then fixed using methanol for 10 min. Cells were stained with 0.5% crystal violet for 30 min and then washed with tap water.

Western blot analysis

G6PD, HIF-1α, HKII, and β-actin antibodies (Cell Signaling Technology, USA) were used for immunoblotting analysis. Cells were grown in 100 mm culture dishes, washed with PBS, scraped off in 1 mL PBS, and collected by centrifugation (1000 rpm, 3 min). The collected cells were lysed on ice for 30 min in RIPA buffer containing 1 mM β-glycerophosphate, 5 mM potassium-fluoride, 0.1 mM sodium orthovanadate, and protease inhibitor cocktails. Cell lysate, 20 μg total, was resolved on a sodium dodecyl sulfate (SDS) polyacrylamide gel, transferred to a polyvinylidene difluoride (PVDF) membrane, and then subjected to western blot analyses using an enhanced chemiluminescence (ECL) system. Experiment results were obtained using a Microchemi™ imaging system (Bio-Imaging System, Israel).

ROS analysis

The Muse Oxidative Stress Reagent Kit (Millipore, USA) was used to analyze cellular reactive oxygen species (ROS) generated after 2 h of cell treatment with 2 μg/mL A-AMF.

Statistical analysis

Data were presented as the mean ± SD of at least three independent experiments. Student's *t*-test or one-way analysis of variance (ANOVA). F-test analyzed associations between the groups. All statistical differences were deemed significant at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Previously, we reported that AMFs could selectively inhibit the proliferation of cancer cells [6, 7]. Here, we investigated the proliferation of melanoma cells following treatment with different AMFs cloned from various sources, such as pancreatic AsPC-1 (As-AMF, Genbank MW664917), prostatic DU145 (D-AMF, Genbank MW664916), cervical HeLa (H-AMF, Genbank KY379509), hepatic HepG2 (Hg-AMF, Genbank MW664918), ovarian SKOV3 (S-AMF, MW664920), breast MCF-7 (M-AMF, Genbank MW664919), colorectal HT-29 (Ht-AMF, Genbank MT843569), and lung A549 (A-AMF, Genbank BC004982) cancer cells. The study revealed that melanoma cells were significantly inhibited by treatment with 2 μg/mL A-AMF and H-AMF, while B16F10 cells seemed to be highly susceptible than SKMEL-2 cells to different types of AMFs (Fig. 1a). In addition, the potency of A-AMF was noticed in a dose-dependent manner (Fig. 1b). GPI is essential for both the glycolytic and pentose phosphate pathway (PPP). It has been well documented that AMF binding to its receptor AMFR/gp78 activates the PI3K/AKT pathway to promote glucose flux into the glycolytic pathway and PPP [17]. Overexpressed G6PD and a higher degree of PPP flux are frequently observed in cancers [18]. Using SKMEL-2 cells, we quantified G6PD, hexokinase II (HK2), GPI, and aldolase A (ALDA) mRNA expression. A significant decrease was detected in G6PD mRNA expression (Fig. 2) following treatment with A-AMF for 24 h, whereas no change was detected in the mRNA level of glycolytic components, like HK2, GPI, and ALDA. In addition, decreased G6PD protein expression in SKMEL-2 as well as B16-F10 cells (Fig. 3a) suggested that A-AMF-induced melanoma cell growth inhibition was mainly through suppression of G6PD expression followed by diminished PPP flux. Considering the importance of PPP flux which produces various metabolites essential for proliferation, malignant progression, and chemo- and radio-resistance [19], our finding might provide a novel strategy to treat melanoma. HIF-1α is a master transcriptional regulator of adaptive responses to hypoxia. It has a crucial role in adapting cells to hypoxia by regulating the expression of many genes, including glycolysis-related genes [20]. It was determined that A-AMF can cause decreased HIF-1α mRNA expression in SKMEL-2 cells (Fig. 2) and decreased protein synthesis in SKMEL-2 as well as B16-F10 cells (Fig. 3a). Taken together with the finding of elevated ROS generation in SKMEL-2 cells (Fig. 3b), PPP flux regulation through A-AMF-mediated HIF-1α/G6PD expression regulation may be a

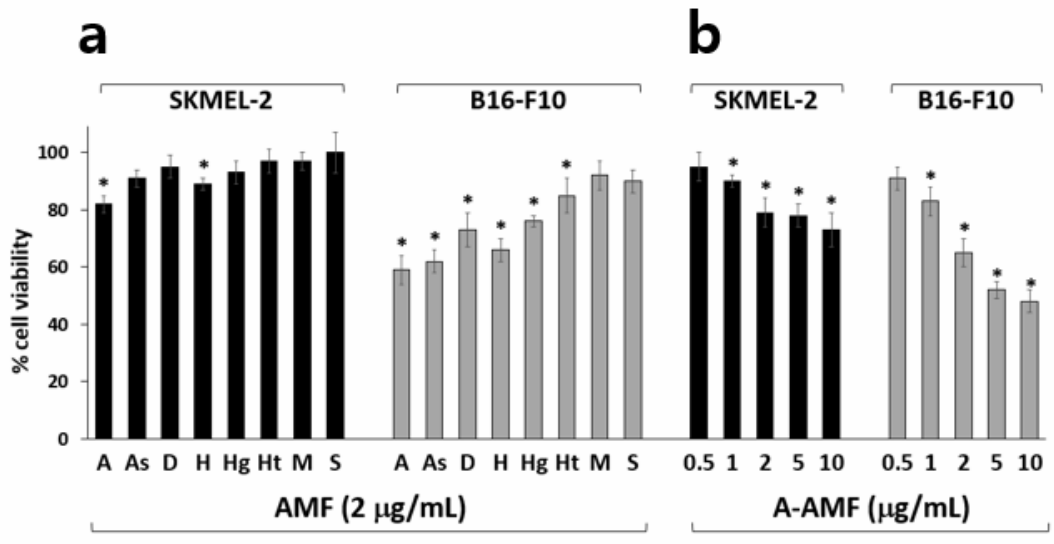

Fig. 1. The effect of AMF on the proliferation of melanoma cells. Human SKMEL-2 and mouse B16-F10 melanoma cells were treated with different AMFs (a) and also with A-AMF (b) for 48 h. Cell growth was evaluated by MTT assay and comparatively quantified. Each bar represents the mean of triplicate measurements ± SE, and $^{*}P < 0.05$.

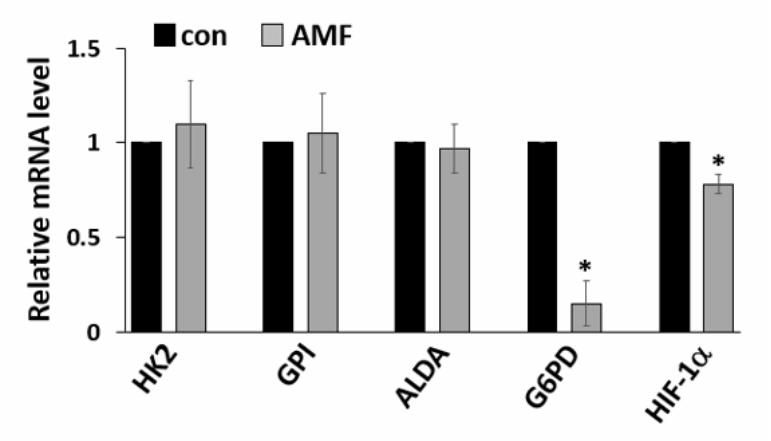

Fig. 2. The effect of A-AMF on mRNA expression in SKMEL-2 cells. After treatment with 2 μg/mL A-AMF of SKMEL-2 cells for 24 h, the relative mRNA expression levels of glycolytic genes were quantified. Each bar represents the mean of triplicate measurements ± SE, and $^{*}P < 0.05$.

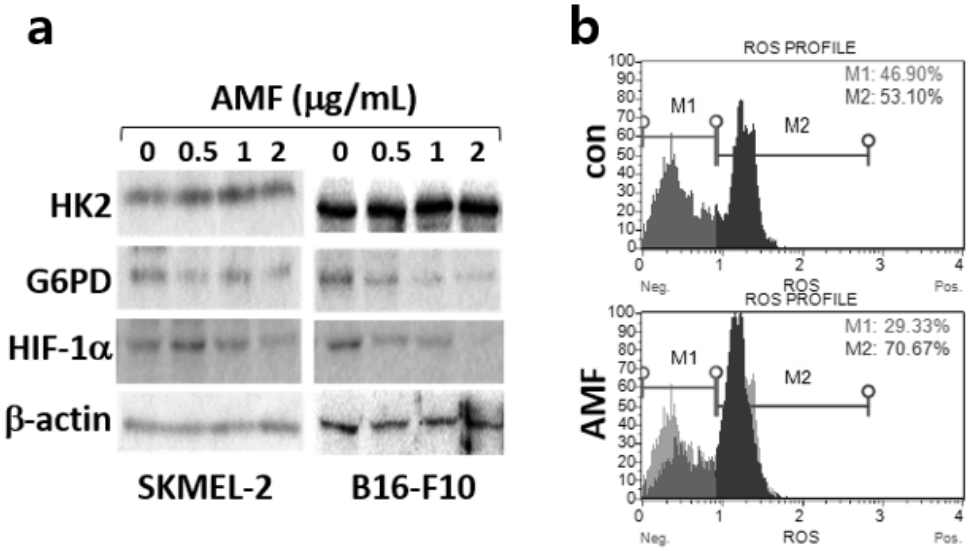

Fig. 3. The effect of A-AMF on protein expression and ROS generation in melanoma cells. After treatment with A-AMF at various concentrations for 24 h, the expression levels of indicated proteins were determined by immunoblot analysis (a). After treatment with 2 μg/mL A-AMF for 2 h, ROS generation in SKMEL-2 cells was monitored (b).

significant cause of diminished melanoma cell growth.

Many phytochemicals have shown to be potential anticancer reagents, but their bioavailability remains in doubt. As one of the many plant-derived agents with anticancer activities, OG is notably cell permeable and highly bioavailable. In this study, OG, even at a concentration as low as 1 μM, was severely effective in controlling SKMEL-2 and B16-F10 melanoma cells (Fig. 4a, 4c). In combined treatments, A-AMF and OG showed a synergistic effect in limiting melanoma cell proliferation (Fig. 4b, 4d). The tumor microenvironment may be stressful due to depleted nutrients, growth factors, and space availability. In a cell stress tolerance assay, it was observed that A-AMF and OG in combination could diminish the viability of melanoma cells more quickly than A-AMF or OG alone (Fig. 5). This study also demonstrated the synergistic activity of A-AMF and OG in the control of melanoma cells.

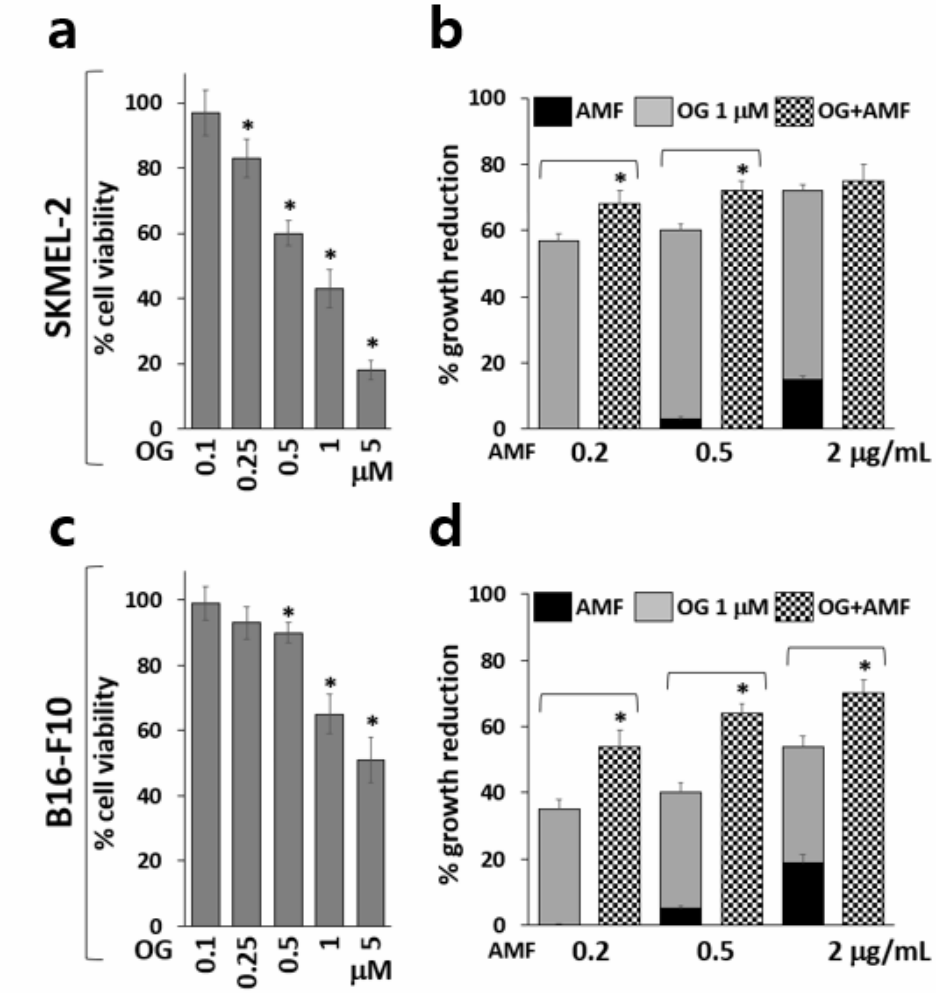

Fig. 4. The effects of OG and A-AMF on the proliferation of melanoma cells. SKMEL-2 cell growth was determined after treatment with OG alone (a) and in combination with A-AMF (b). Similarly, B16-F10 cells were treated with OG alone (c) and in combination with A-AMF (d). Each bar represents the mean of triplicate measurements ± SE, and $^{*}P < 0.05$.

Earlier, AMF was indicated as a potential trigger of cell competition, which could determine cancer cell fate [6]. This was further corroborated by the differential activity of AMF presented in this study. G6PD, as a housekeeping enzyme, is indispensable for growth and development. In cancer cells, increased G6PD activity and elevated NADPH, fatty acid, and nucleic acid production are closely associated with the activation of diverse pro-oncogenic signals such as Ras, Src, STAT, and PI3/AKT [21, 22]. In this regard, A-AMF-mediated G6PD expression regulation through downregulation of HIF-1α expression could be highly significant. Meanwhile, extracellular AMF works in an autocrine and paracrine manner, presumably without being discriminated by its producer cancer cells or other cancer cells. Since the cytosolic GPI enzyme is essential for cell growth and maintenance, resistance to AMF originating from any cells and cancerous cells might rarely be brought about in AMF-treated cancer cells [23]. Considering this self-recognition property in addition to selective killing ability and sensitizing effectiveness [24-26], we strongly suggest the use of AMF to treat melanoma with high metabolic plasticity and proficient resistance development.

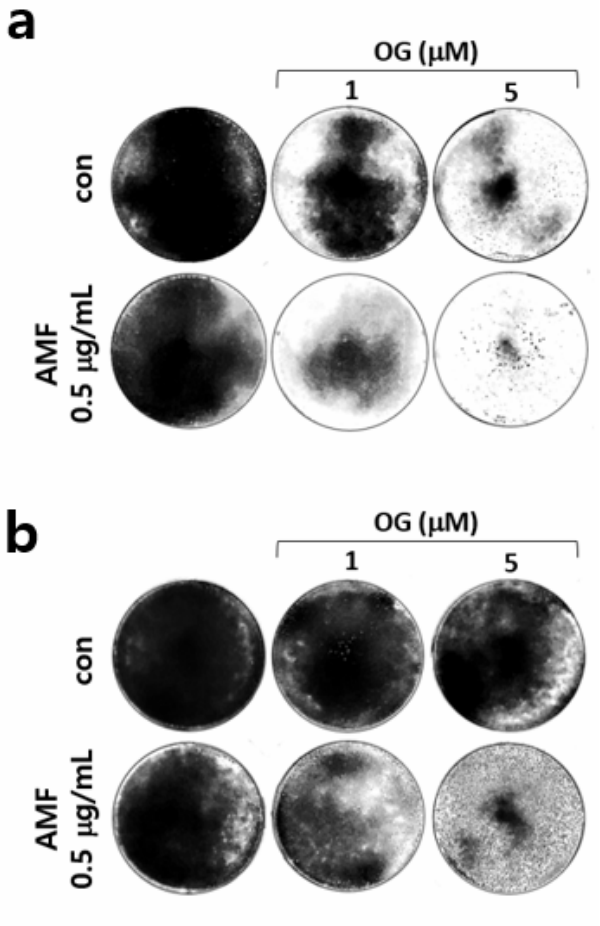

Fig. 5. The effect of A-AMF and OG on melanoma cells at 100% confluence. Confluently cultivated SKMEL-2 cells (a) and B16-F10 cells (b) in a 12 well culture plate were treated with A-AMF and OG alone or in combination for 36 h and the results were monitored after crystal violet staining.

CONCLUSION

AMF and OG might become am attractive combination to increase melanoma treatment efficacy while lowering each other's dose. Such combination may provide a promising alternative for melanoma therapy.

ACKNOWLEDGEMENT

This work was supported by Daegu Catholic University's Intramural Research Fund 2020.

CONFLICT OF INTEREST STATEMENT

The authors have no potential conflict of interest to report.

REFERENCES

1. Long. G. V., Eroglu, Z., Infante, J., Patel, S., Daud, A., Johnson, D. B., Gonzalez, R., Kefford, R., Hamid, O., Schuchter, L., Cebon, J., Sharfman, W., McWilliams, R., Sznol, M., Redhu, S., Gasal, E., Mookerjee, B., Weber, J. and Flaherty K. T. 2018, J. Clin. Oncol., 36, 667.
2. Griffin, M., Scotto, D., Josephs, D. H., Mele, S., Crescioli, S., Bax, H. J., Pellizzari1, G., Wynne, M. D., Nakamura, M., Hoffmann, R. M., Ilieva, K. M., Cheung, A., Spicer, J. F., Papa, S., Lacy, K. E. and Karagiannis, S. N. 2017, Oncotarget, 8, 78174.
3. Czarnecka, A. M., Bartnik, E., Fiedorowicz, M. and Rutkowski, P. 2020, Int. J. Mol. Sci., 21, 4576.
4. Snajdauf, M., Havlova, K., Vachtenheim Jr, J., Ozaniak, A., Lischeke, R., Bartunkova, J., Smrz, D. and Strizova, Z. Front. Mol. Biosci., 8, 628332.
5. Lezhnin, Y. N., Kravchenko, Y. E., Frolova, E. I., Chumakov, P. M. and Chumakov, S. P. 2015, Mol. Biol., 49, 231.
6. Park, H. S. and Jeoung, N. H. 2020, Biochem. Bioph. Res. Co., 525, 557.
7. Jeoung, N. H., Jo, A. L. and Park, H. S. 2021, Biosci. Biotechnol. Biochem., 85, 1711.
8. Kojic, L. D., Wiseman, S. M., Ghaidi, F. and Joshi, B. 2008, PLoS One, 3, e3597.
9. Jiang, W. G., Raz, A., Douglas-Jones, A. and Mansel, R. E. 2006, J. Histochem. Cytochem., 54, 231.
10. Kathagen-Buhmann, A., Maire, C. L., Weller, J., Shulte, A., Matschke, J., Holze, M., Ligon, K. L., Westphal, M. and Lamszus, K. 2018, Neuro. Oncol., 20, 1594.
11. Gong, W., Jiang, Y., Wang, L., Wei, D., Yap, J., Huang, S., Fang, S. and Xie, K. 2005, Clin. Cancer. Res., 11, 5778.
12. Ma, Y. T., Xing, X. F., Dong, B., Cheng, X., Guo, T., Du, H., Wen, X. and Ji, J. 2018, Cancer Manag. Res., 10, 4969.
13. Wolf, V. G., Bonacorsi, C., Raddi, M. S. G., da Fonsexa, L. and Ximenes, V. F. 2017, Food Funct., 8, 2500.
14. Tang, N. T. K. and Yoo, H. 2020, Int. J. Oral. Biol., 45, 218.

15. Serrano, A., Palacios, C., Roy, G., Cespon, C., Villar, M. L., Nocito, M. and Gonzlez-Porque, P. 1998, Arch. Biochem. Biophys., 350, 49.
16. Chua, K. V., Fan, C. S., Chen, C., Chen, L., Hsieh, S. and Huang, T. 2020, Cells, 9, 91.
17. Jiang, P., Wenjing, D. and Yang, X. 2013, Cell Cycle, 12, 3720.
18. Jiang, P., Wenjing, D. and Mian, W. 2014, Protein Cell, 5, 592.
19. Phan, L. M., Yeung, S. C. and Lee, M. H. 2014, Cancer Biol. Med., 11, 1.
20. Huang, R. and Zhou, P. K. 2020, Radiat. Med. Prot., 1, 7.
21. Yang, H. C., Wu, Y. H., Yen, W. C., Liu, H., Whang, T., Stern, A. and Chiu, D. T. 2019, Cells, 8, 1055.
22. Pai, S. G., Carneiro, B. A., Mota, J. M., Fosta, R., Leite, C. A., Barroso-Sousa, R., Kaplan, J. B., Chae, Y. K. and Giles, F. J. 2017, J. Hematol. Oncol., 10, 101.
23. Serna, N., Sanchez-Garcia, L., Unzueta, U., Diaz, R., Vazquez, E., Mangues, R. and Villaverde, A. 2018, Trends Biotechnol., 36, 318.
24. Tamori, Y. and Deng, W. M. 2011, J. Genet. Genomics, 38, 484.
25. Portela, M., Casas-Tinto, S., Rhiner, C., Lopez-Gay, J. M., Dominguez, O., Soldini, D. and Moreno, E. 2010, Dev. Cell, 19, 562.
26. Rhiner, C., Lopez-Gay, J. M., Soldini, D., Casas-Tinto, S., Martin, F. A., Lombardia, L. and Moreno, E. 2010, Dev. Cell, 18, 985.